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(54) **COMPOSITIONS COMPRISING PERFLUOROOCTANOIC ACID**

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A61K 31/513	(2006.01)
A61K 31/395	(2006.01)
A61K 31/69	(2006.01)
A61P 35/00	(2006.01)
C07C 53/21	(2006.01)
A61K 31/436	(2006.01)

(52) **U.S. Cl.** **514/34**; 554/226; 514/558; 514/49; 514/263.4; 514/291; 514/274; 514/183; 514/64

(57) **ABSTRACT**

There is provided compositions comprising perfluorooctanoic acid (PFOA) or a salt, derivative or variant thereof. There is also provided uses, methods therapeutic systems and combination therapies relating to PFOA.

Figure 1

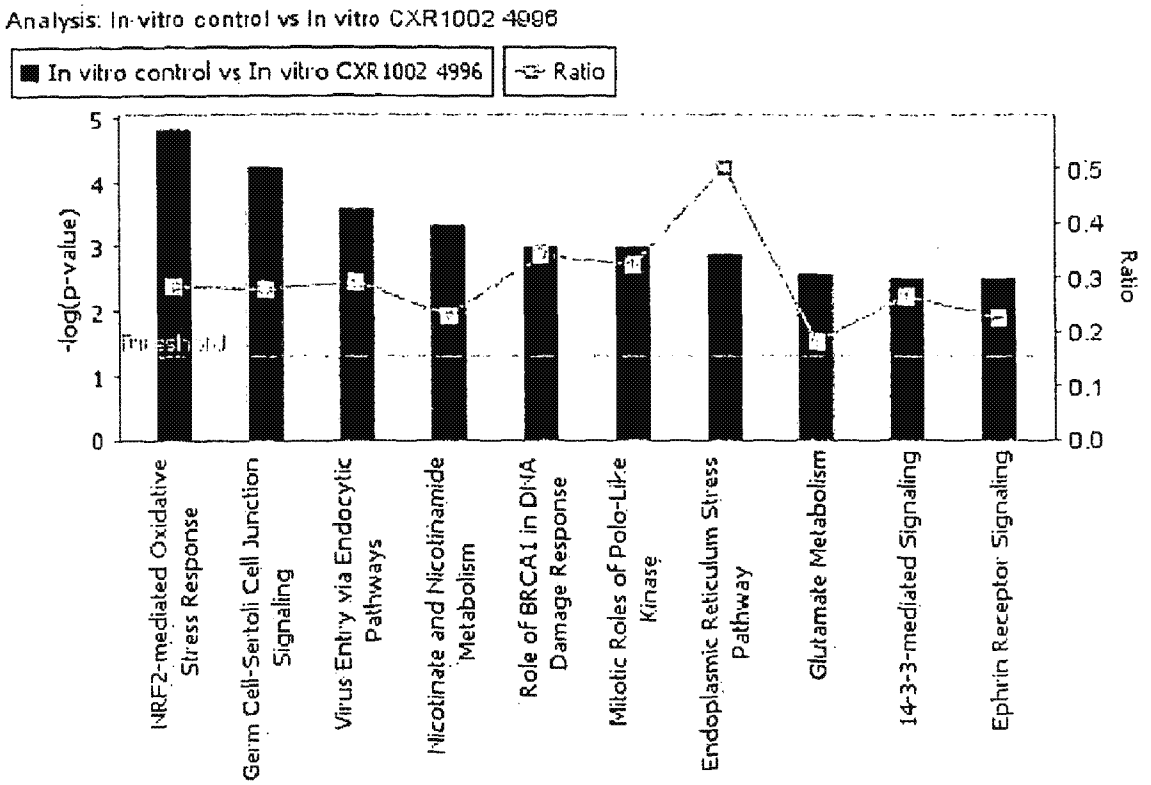


Figure 2

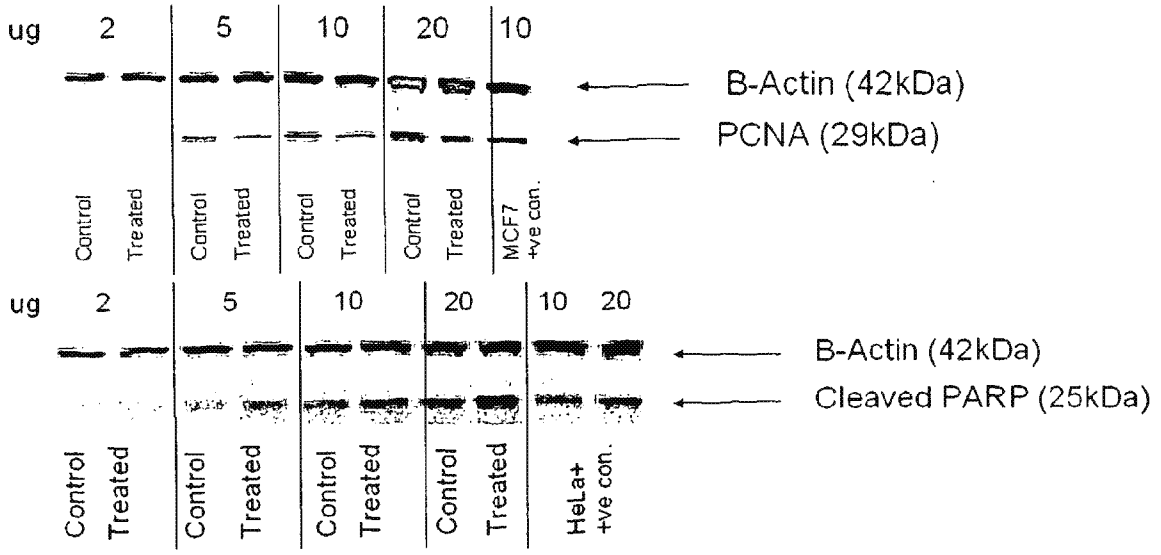

ug
2
5
10
20
10
B-Actin (42kDa)
PCNA (29kDa)
Control
Treated
Control
Treated
Control
Treated
Control
Treated
MCF7 +ve con.
ug
2
5
10
20
10
20
B-Actin (42kDa)
Cleaved PARP (25kDa)
Control
Treated
Control
Treated
Control
Treated
Control
Treated
HeLa+ +ve con.

Figure 3

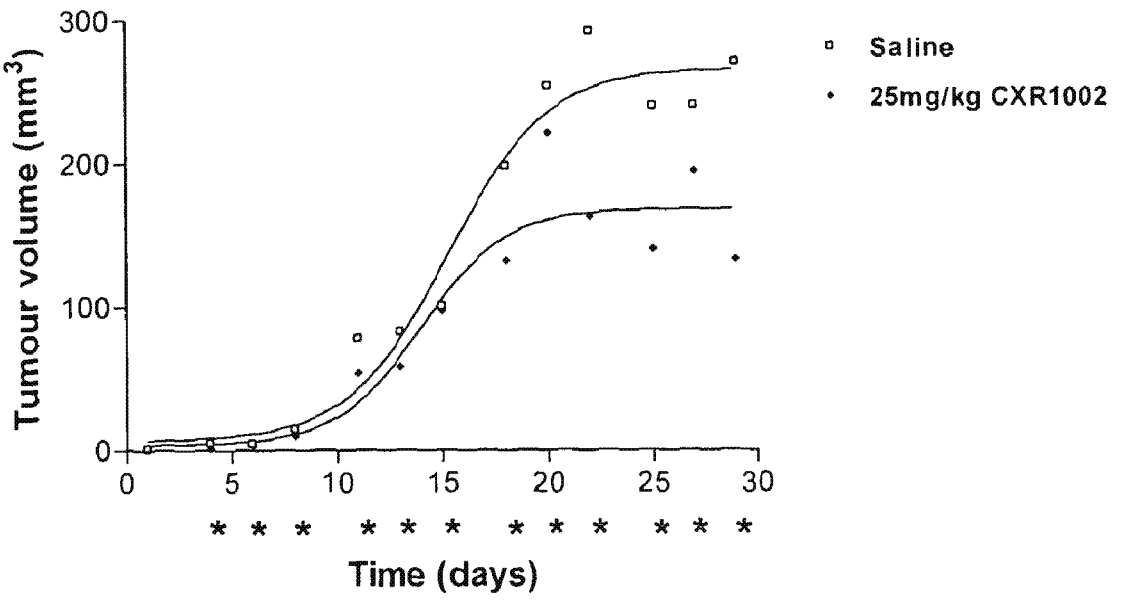

Tumour volume (mm3)
300
200
100
0
0
5
10
15
20
25
30
Time (days)
Saline
25mg/kg CXR1002

Figure 4

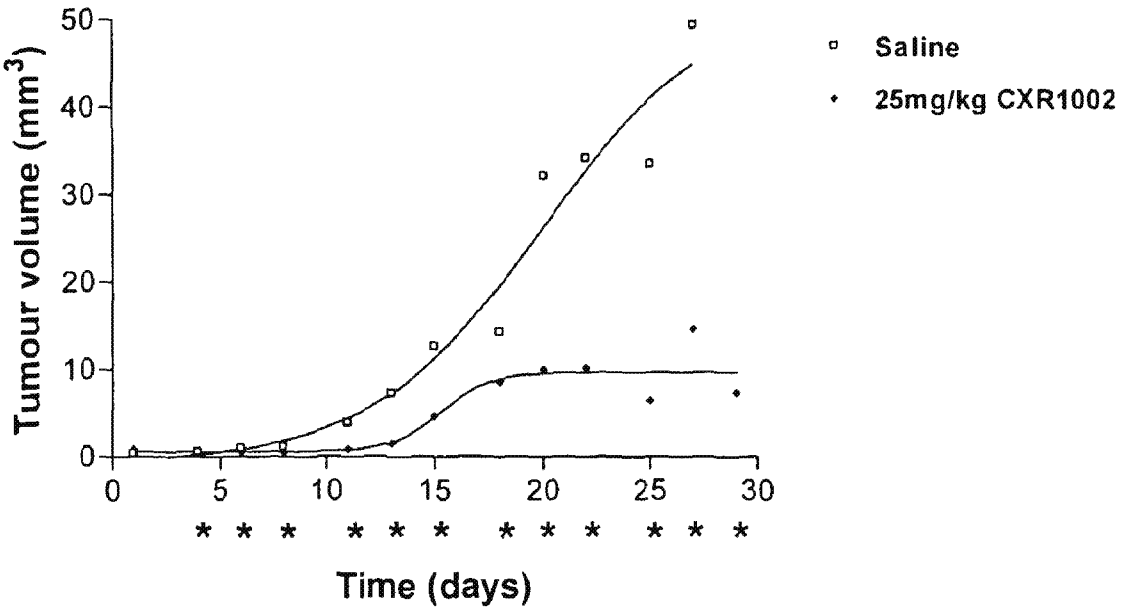

Tumour volume (mm3)
50
40
30
20
10
0
0
5
10
15
20
25
30
Time (days)
Saline
25mg/kg CXR1002

Figure 5

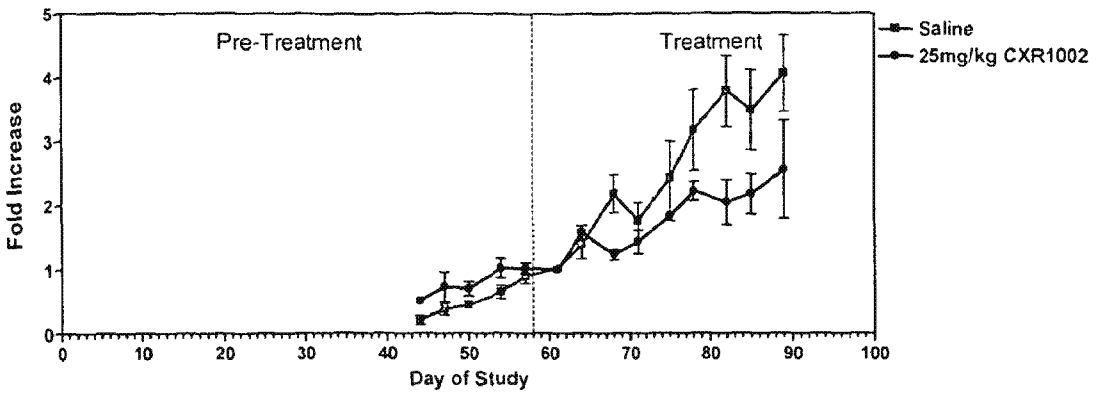


Figure 6

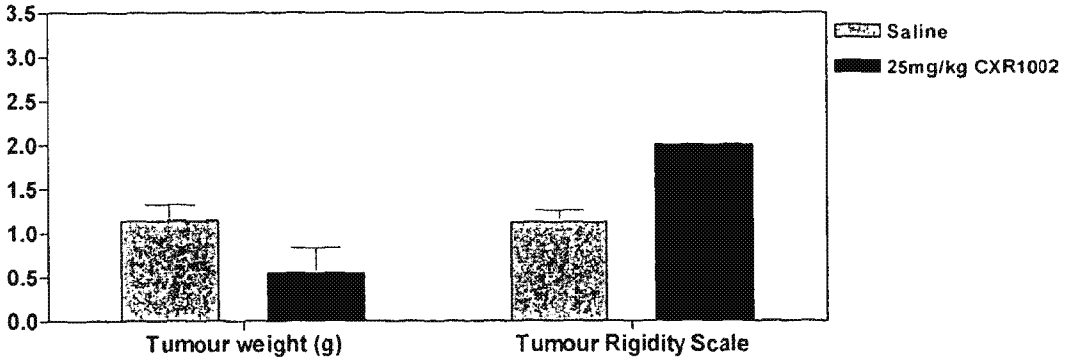


Figure 7

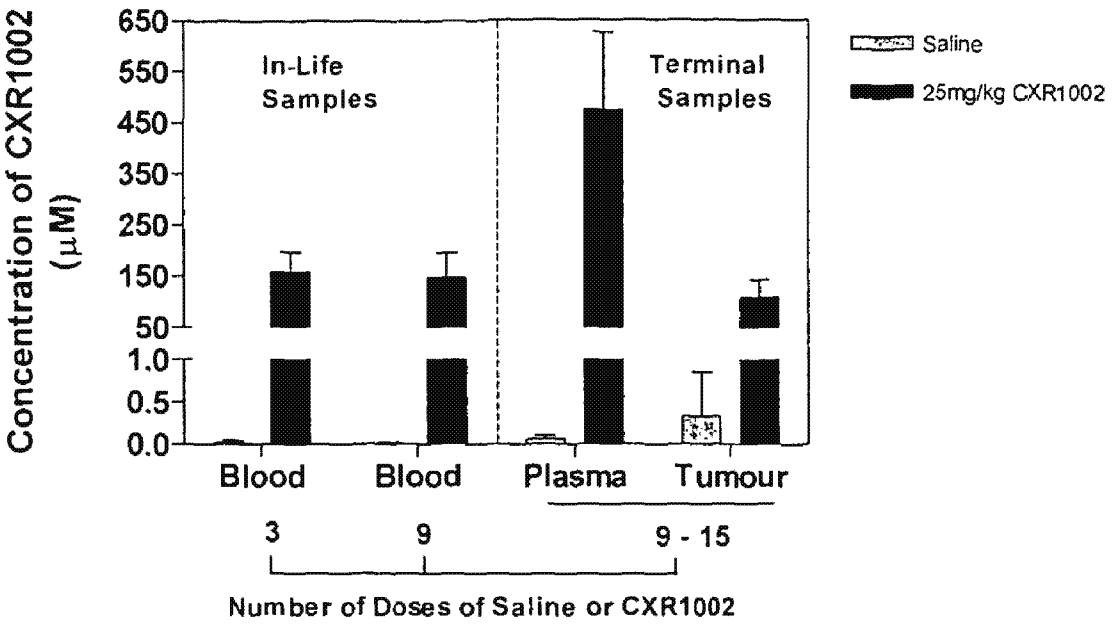

Concentration of CXR1002 (μM)
650
550
450
350
250
150
50
1.0
0.5
0.0
In-Life Samples
Terminal Samples
Saline
25mg/kg CXR1002
Blood
Blood
Plasma
Tumour
3
9
9 - 15
Number of Doses of Saline or CXR1002

Figure 8

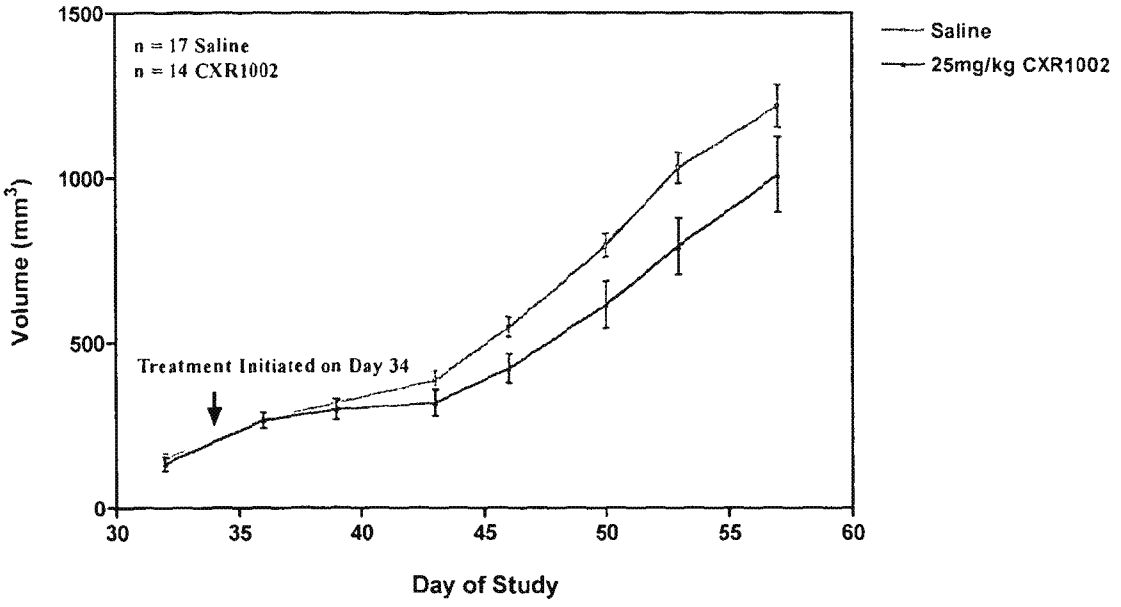


Figure 9

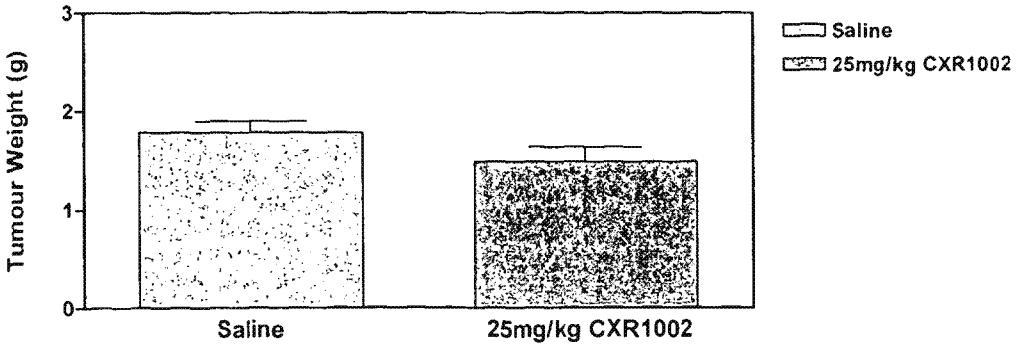


Figure 10

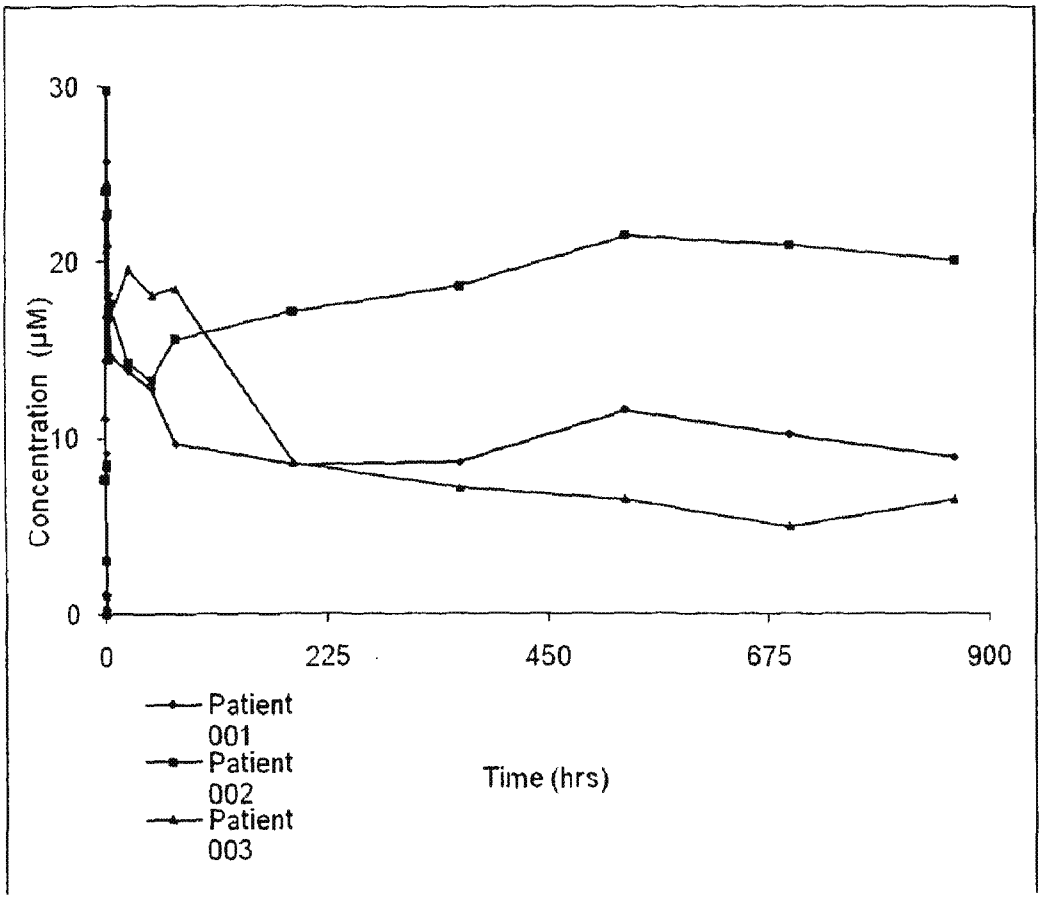

30
20
10
0
Concentration (µM)
0
225
450
675
900
Time (hrs)
Patient 001
Patient 002
Patient 003

Figure 11

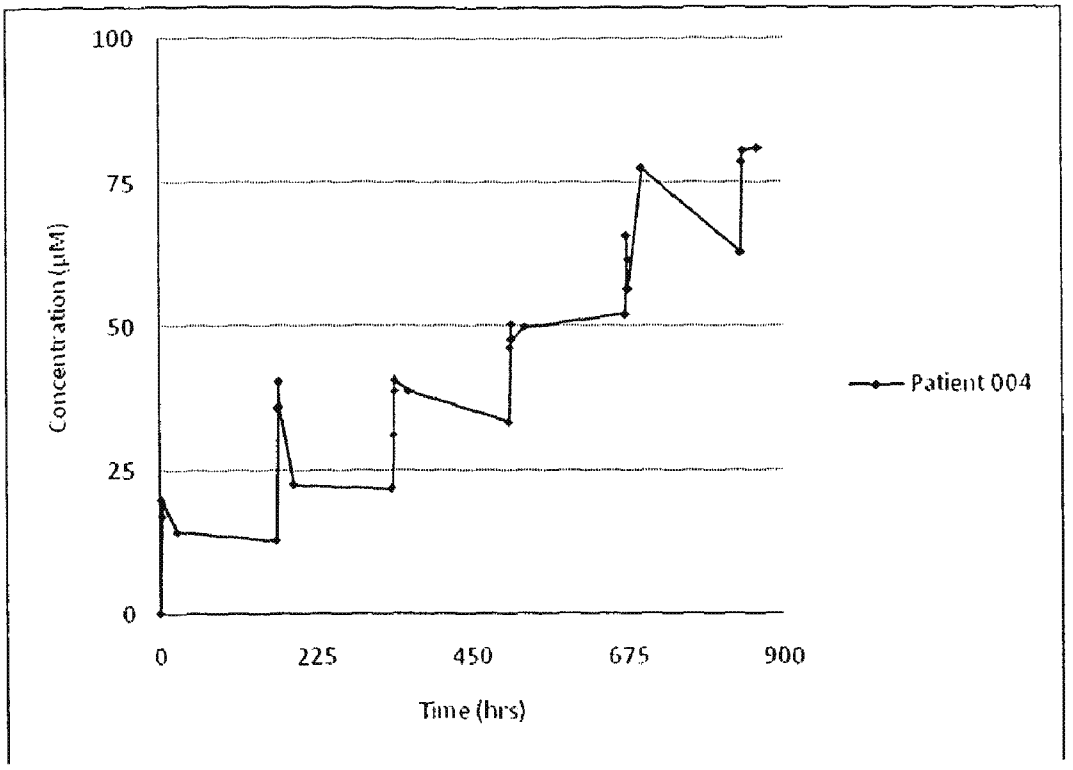

100
75
50
25
0
Concentration (μM)
0
225
450
675
900
Time (hrs)
Patient 004

Figure 12

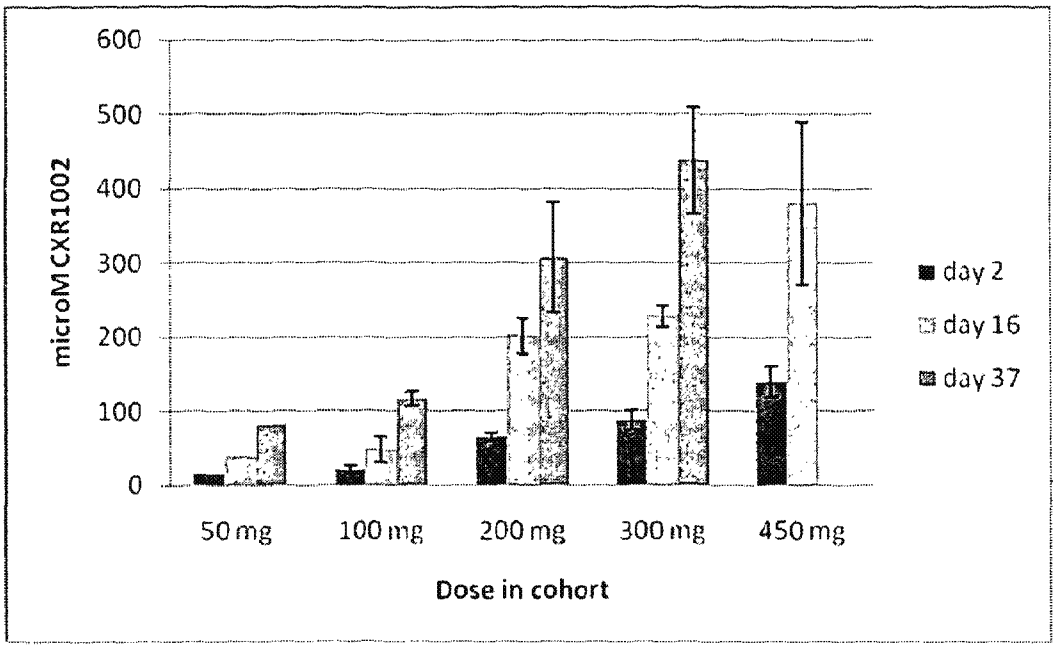

600
500
400
300
200
100
0
microMCXR1002
50 mg
100 mg
200 mg
300 mg
450 mg
Dose in cohort
day 2
day 16
day 37

Figure 13

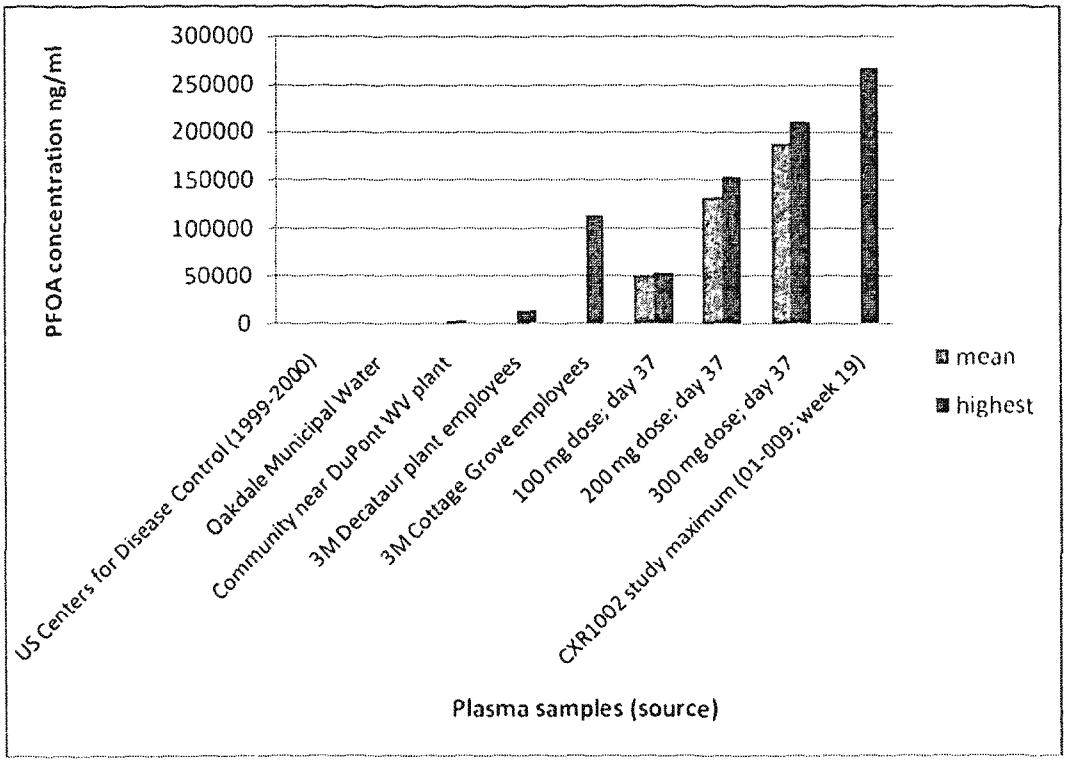

PFOA concentration ng/ml
300000
250000
200000
150000
100000
50000
0
US Centers for Disease Control (1999-2000)
Oakdale Municipal Water
Community near DuPont WV plant
3M Decatur plant employees
3M Cottage Grove employees
100 mg dose; day 37
200 mg dose; day 37
300 mg dose; day 37
CXR1002 study maximum (01-009; week 19)
mean
highest
Plasma samples (source)

Figure 14

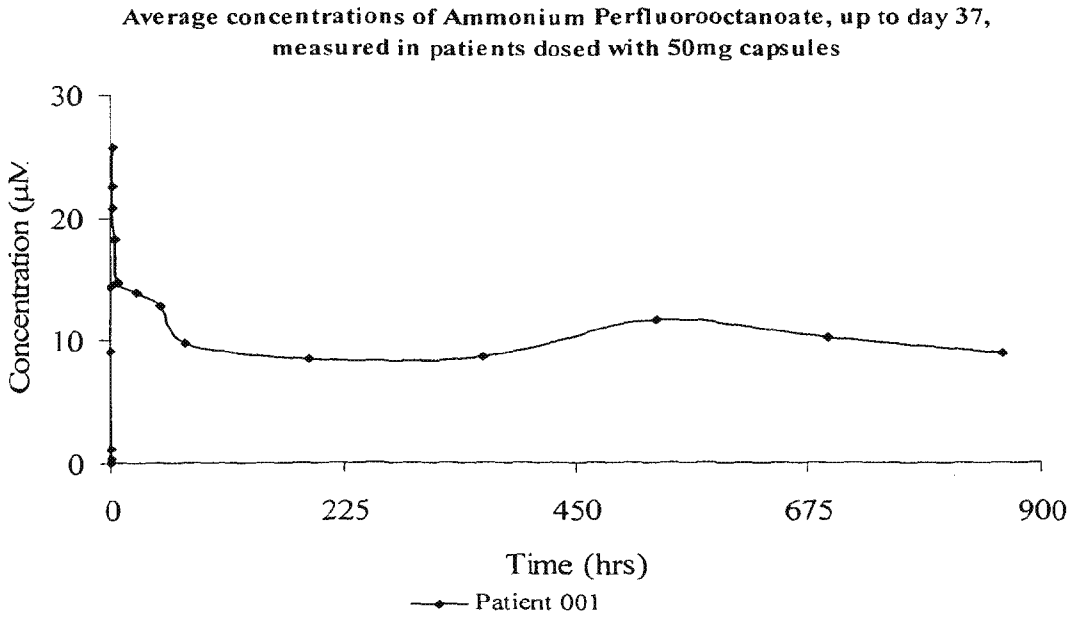


Figure 15

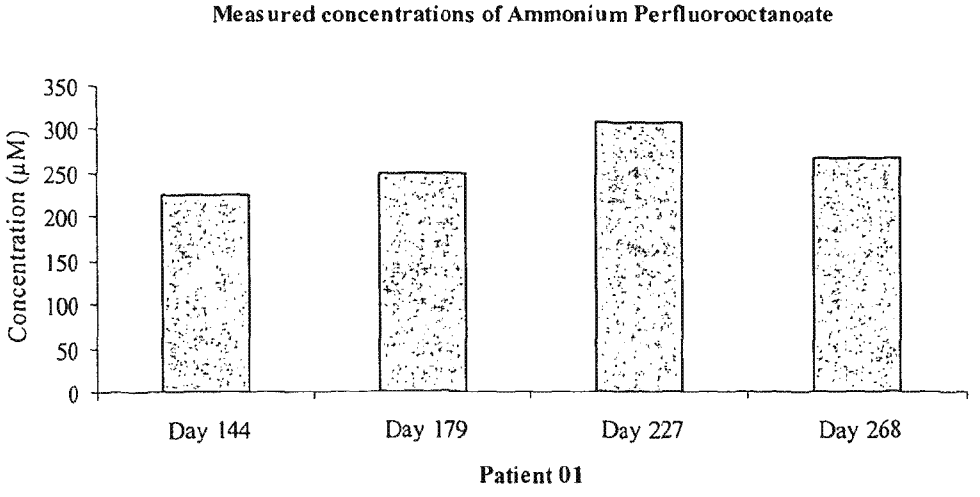
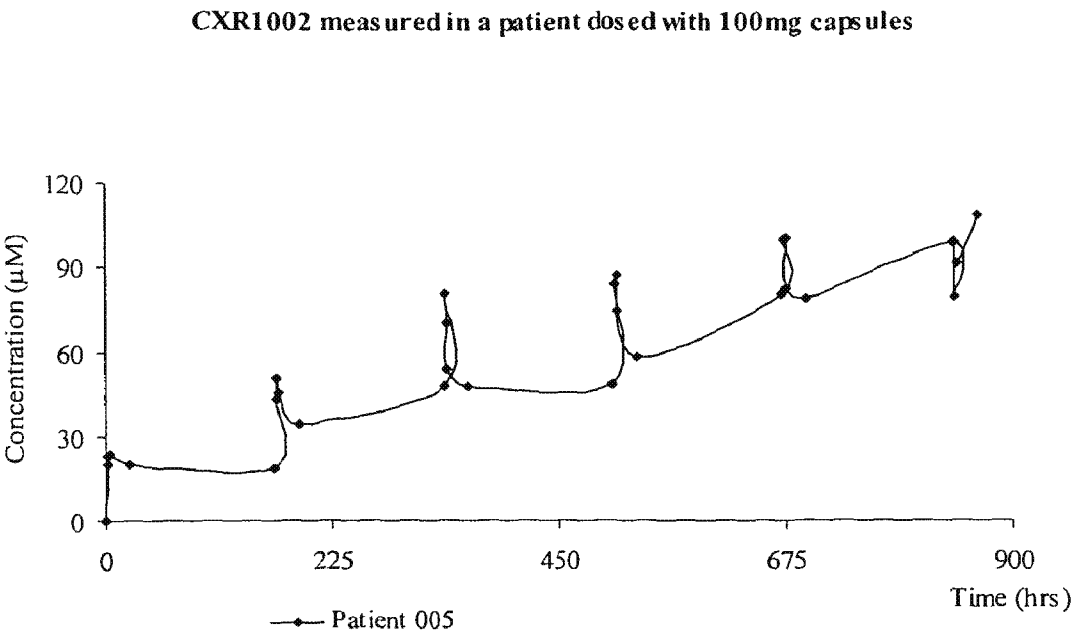


Figure 16

(a)


CXR1002 measured in a patient dosed with 100mg capsules
Concentration (μM)
120
90
60
30
0
0
225
450
675
900
Time (hrs)
Patient 005

(b)

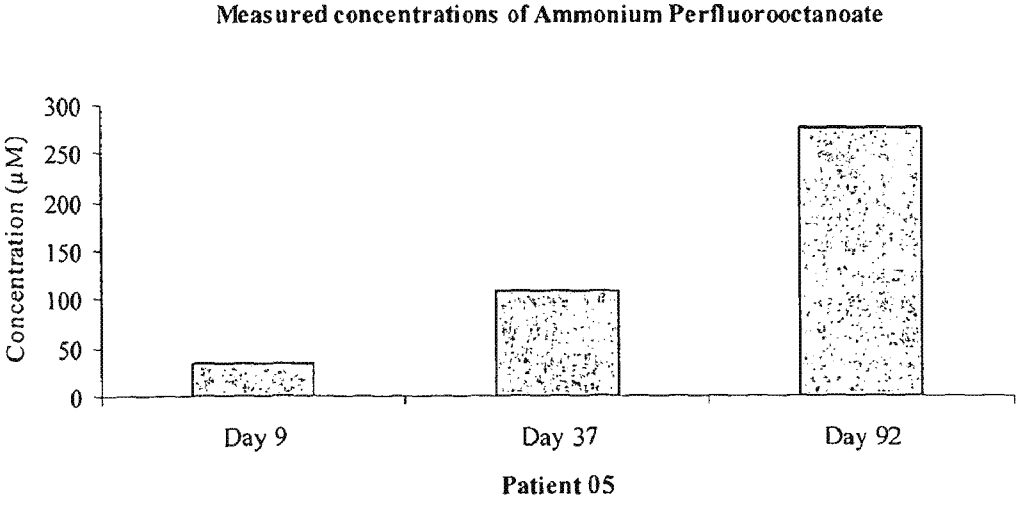

Measured concentrations of Ammonium Perfluorooctanoate
Concentration (μM)
300
250
200
150
100
50
0
Day 9
Day 37
Day 92
Patient 05

Figure 17

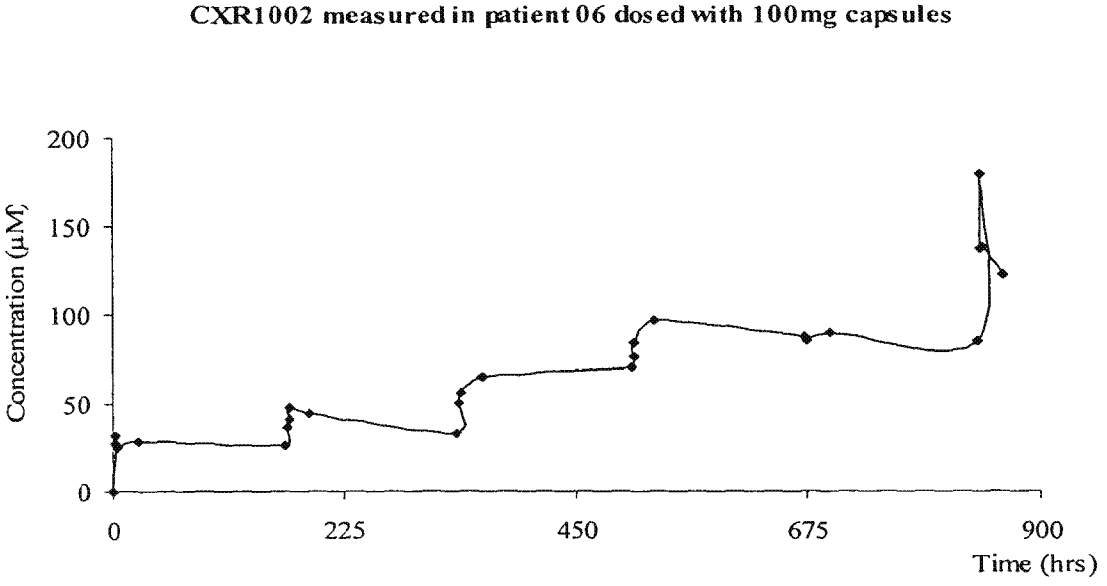


Figure 18

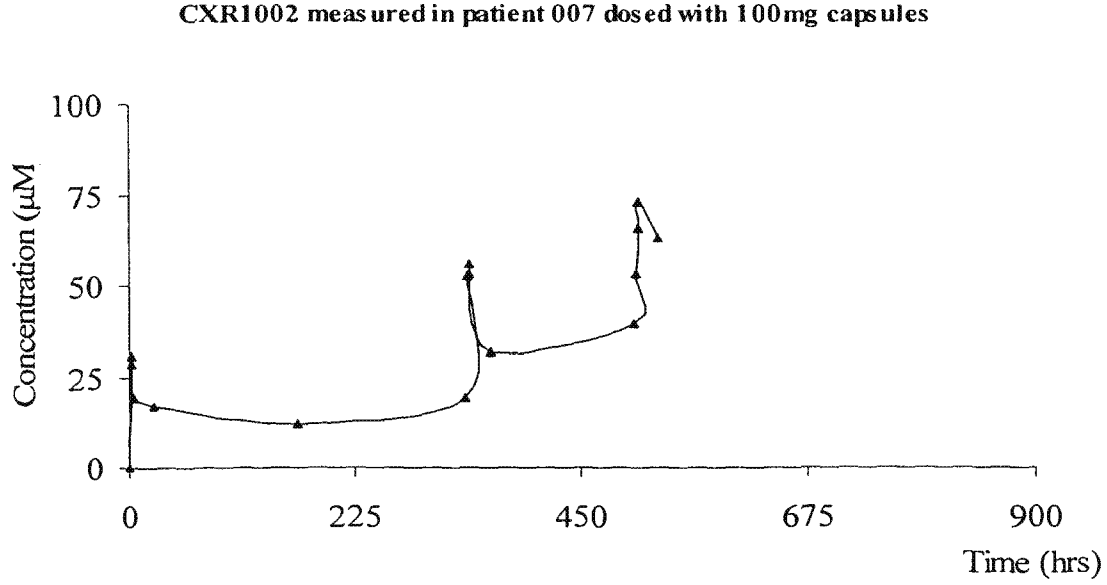


Figure 19

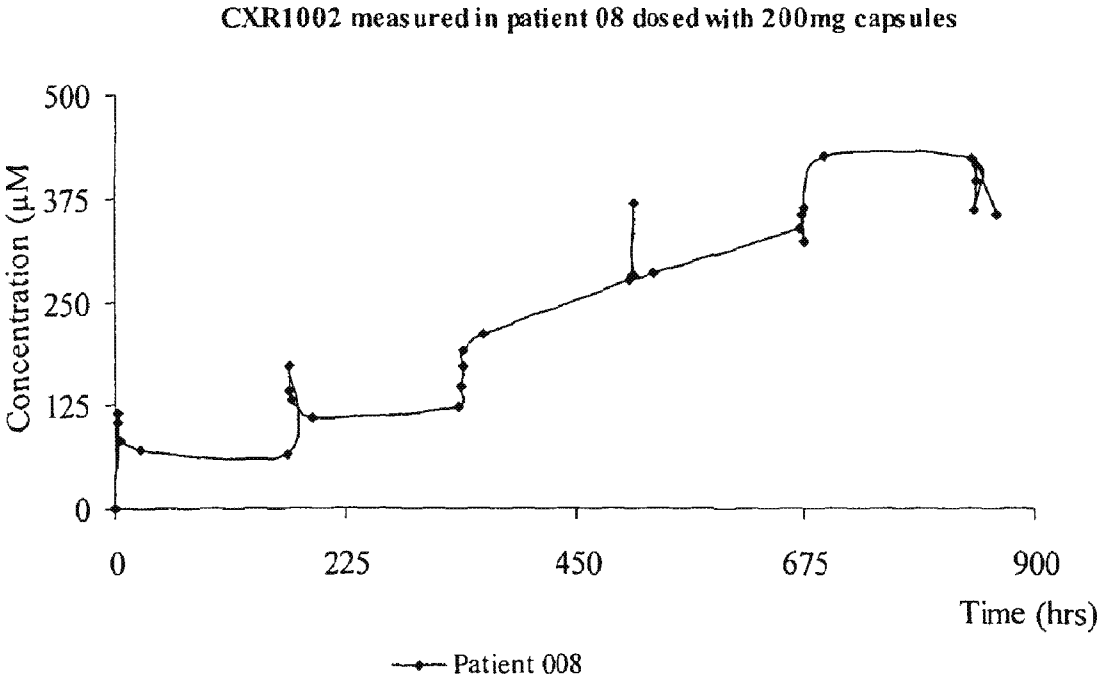
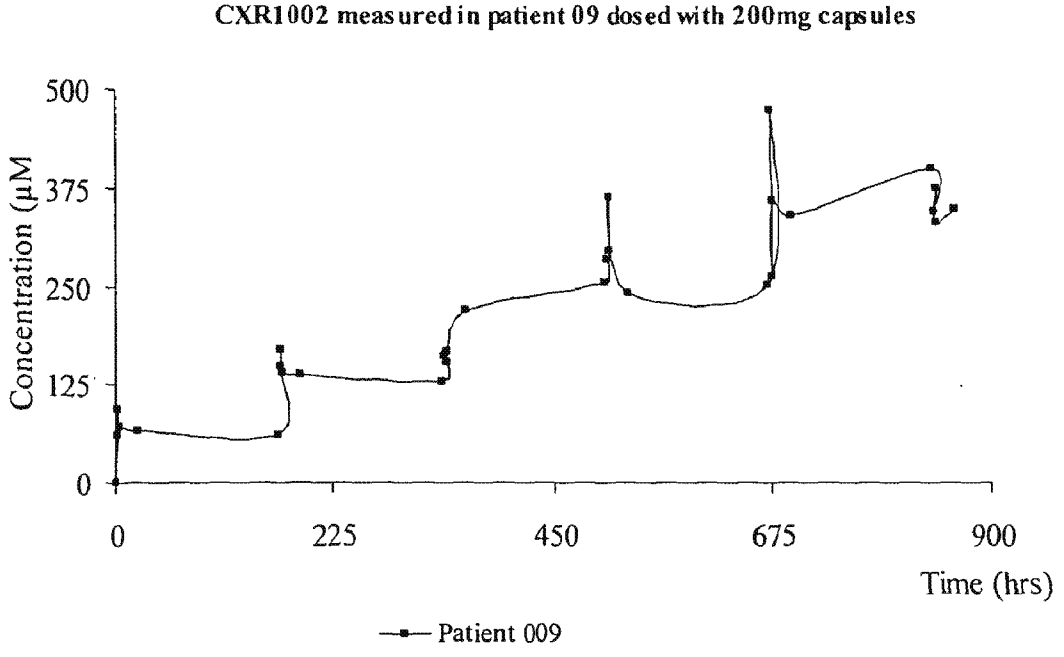
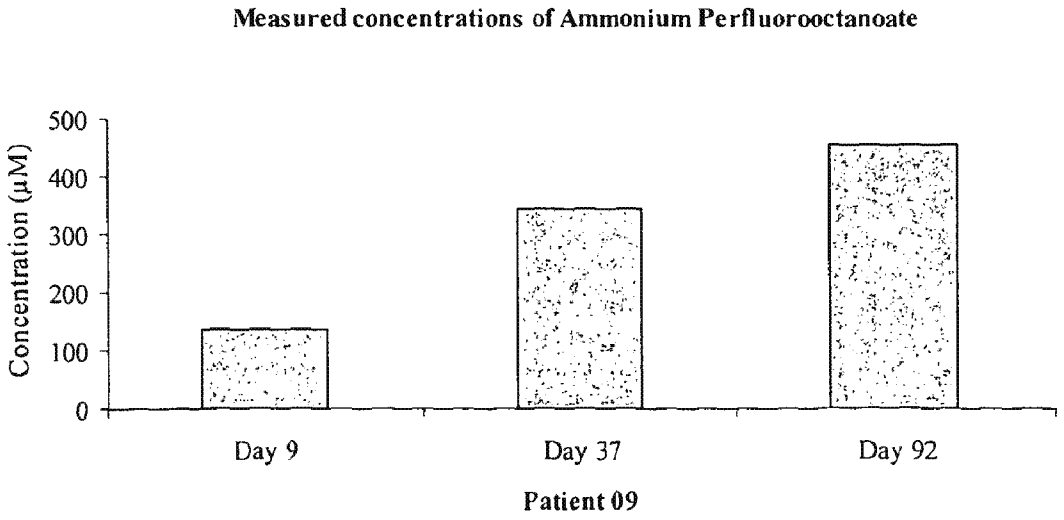

CXR1002 measured in patient 08 dosed with 200mg capsules
Concentration (µM
500
375
250
125
0
0
225
450
675
900
Time (hrs)
Patient 008

Figure 20


CXR1002 measured in patient 09 dosed with 200mg capsules
Concentration (µM)
500
375
250
125
0
0
225
450
675
900
Time (hrs)
Patient 009


Measured concentrations of Ammonium Perfluorooctanoate
Concentration (µM)
500
400
300
200
100
0
Day 9
Day 37
Day 92
Patient 09

Fugure 21

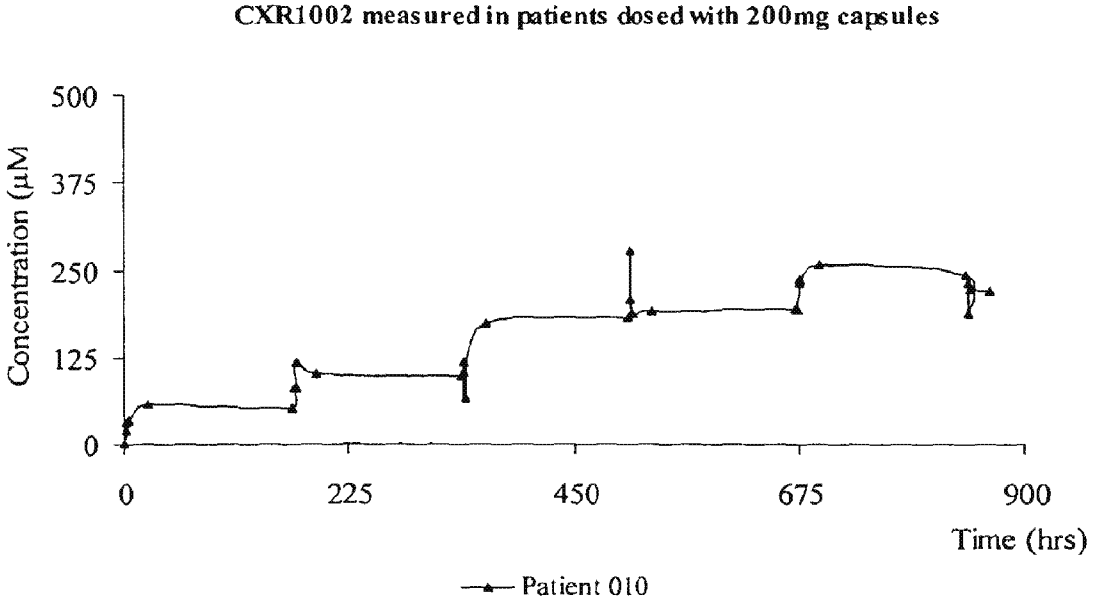


Figure 22

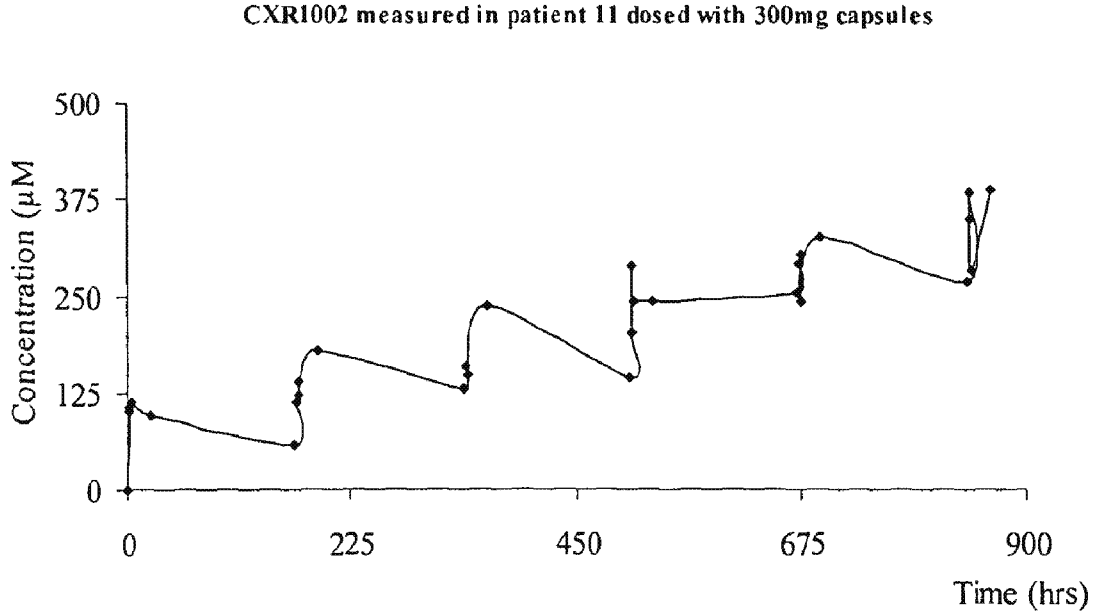


Figure 23

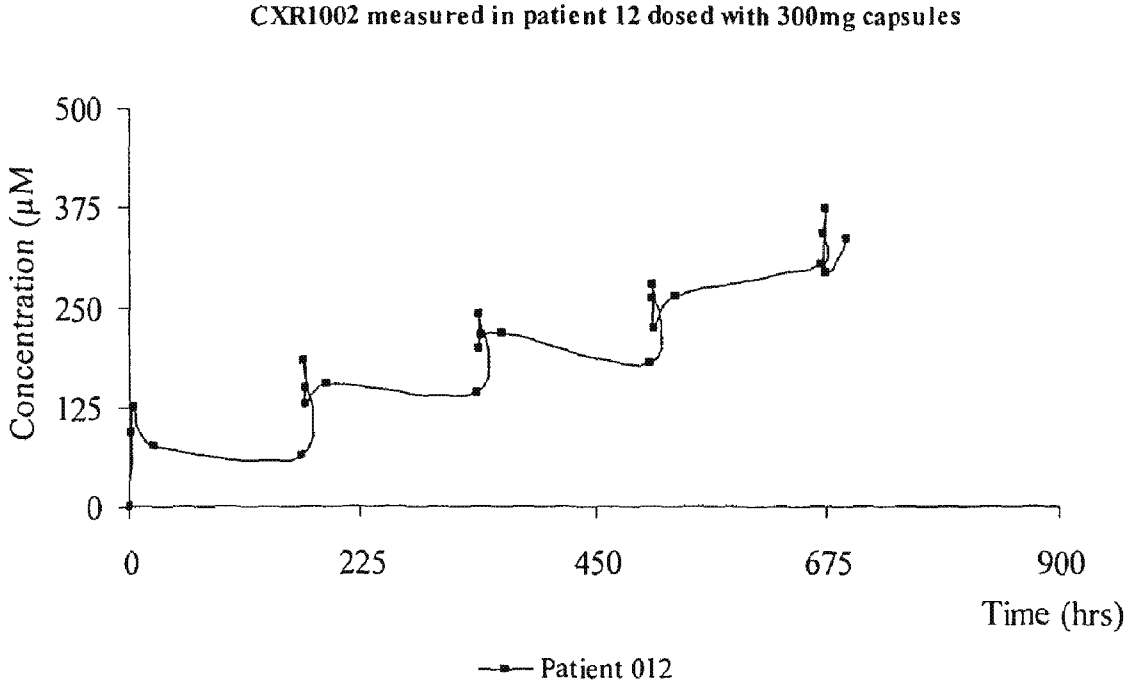

CXR1002 measured in patient 12 dosed with 300mg capsules
Concentration (µM
500
375
250
125
0
0
225
450
675
900
Time (hrs)
Patient 012

Figure 24

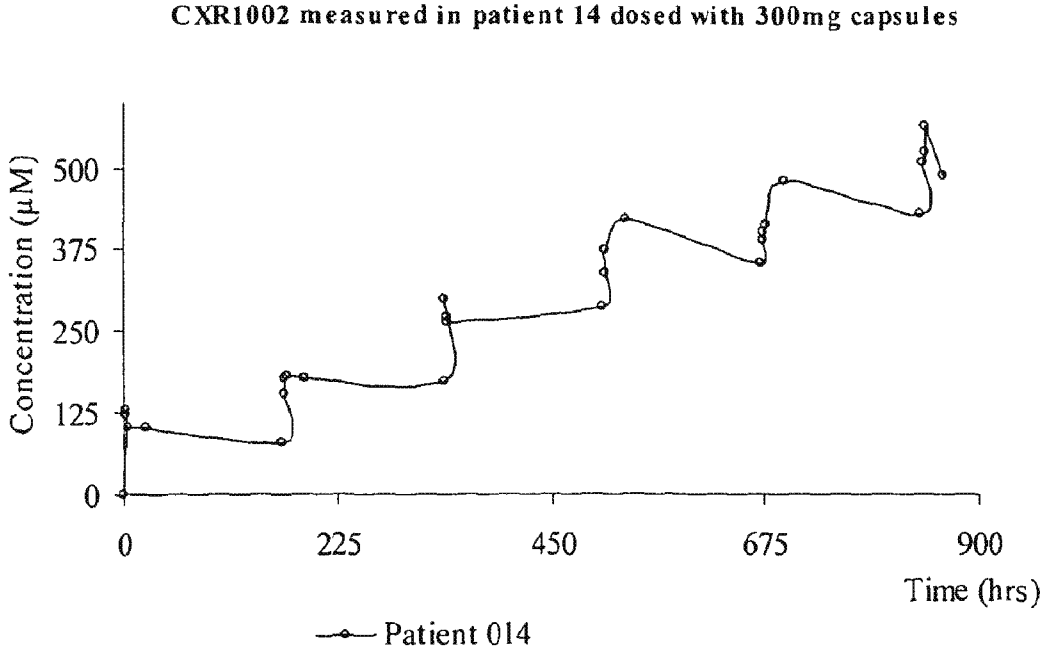


Figure 25

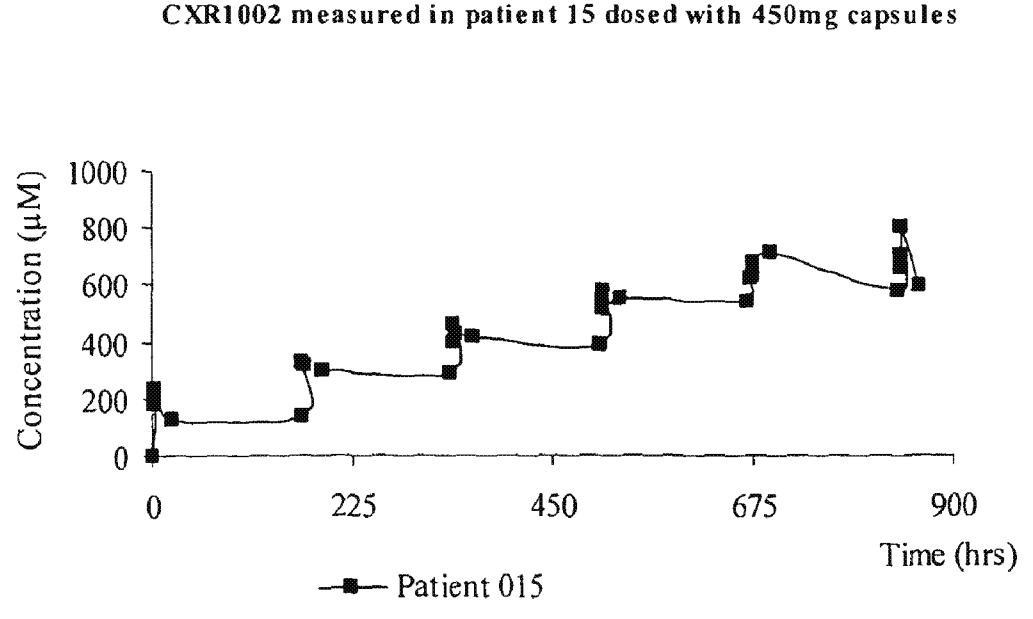


Figure 26

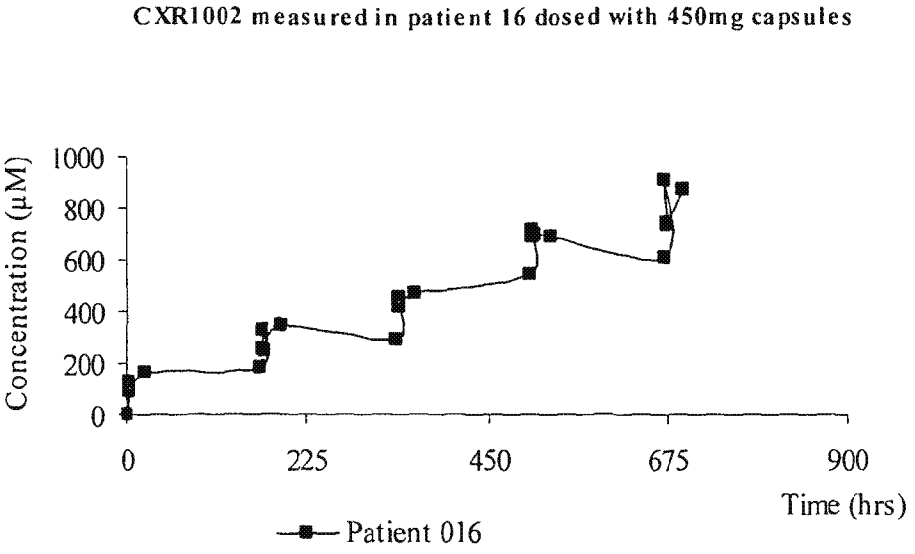


Figure 27

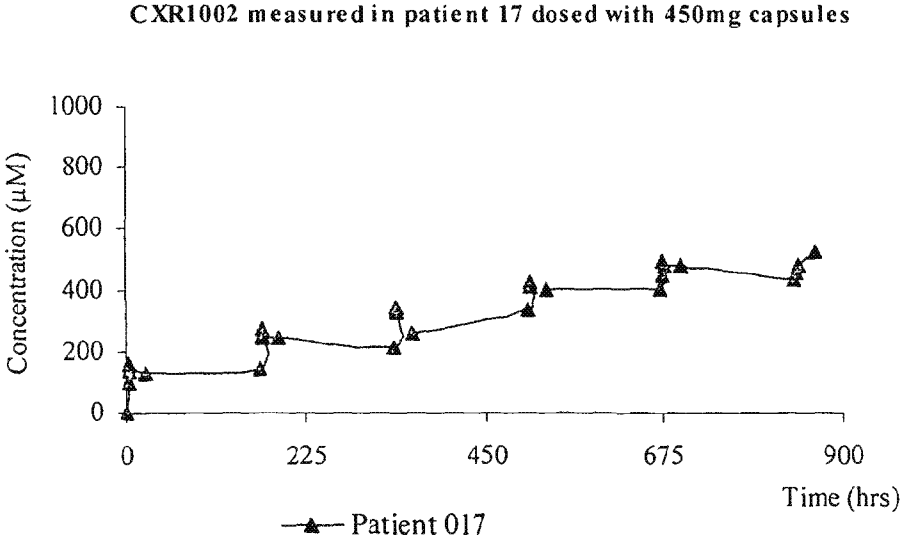


Figure 28

Cell Line	Doxorubicin	Gemcitabine	Geldanamycin	5-FU	Rapamycin	Roscovitine	Docetaxel	Cisplatin
H	Y	G	Y	Y	Y	Y		R
OUMS-27	Y	Y	Y	Y	Y	Y		R
SW1353	Y	Y	Y	Y	Y	Y		R
PANC1	G	G	G	Y	Y	Y		Y
BxPc3	Y	Y	Y	Y	Y	G		Y
HPAFII	Y	Y	Y	Y	Y	Y		Y
Capan2	Y	Y	Y	R	Y	G		Y
SK-OV3	Y	Y	Y	R	G	Y		Y
TOV-21G	Y	G	Y	G	G	Y		Y
OV-90	G	G	G	Y	G	G		Y
OVCAR-3	Y	G	Y	R	G	Y		Y
PC3	G	G	G	Y	R	G		Y
CACO2	Y	Y	Y	Y	Y	Y		R
MDA-MB-157	G	G	Y	G	R	Y		Y
HepG2	Y	Y	G	Y	G	G		Y

Figure 29

Pancreatic Cell Lines treated with CXR 1002 & Doxorubicin

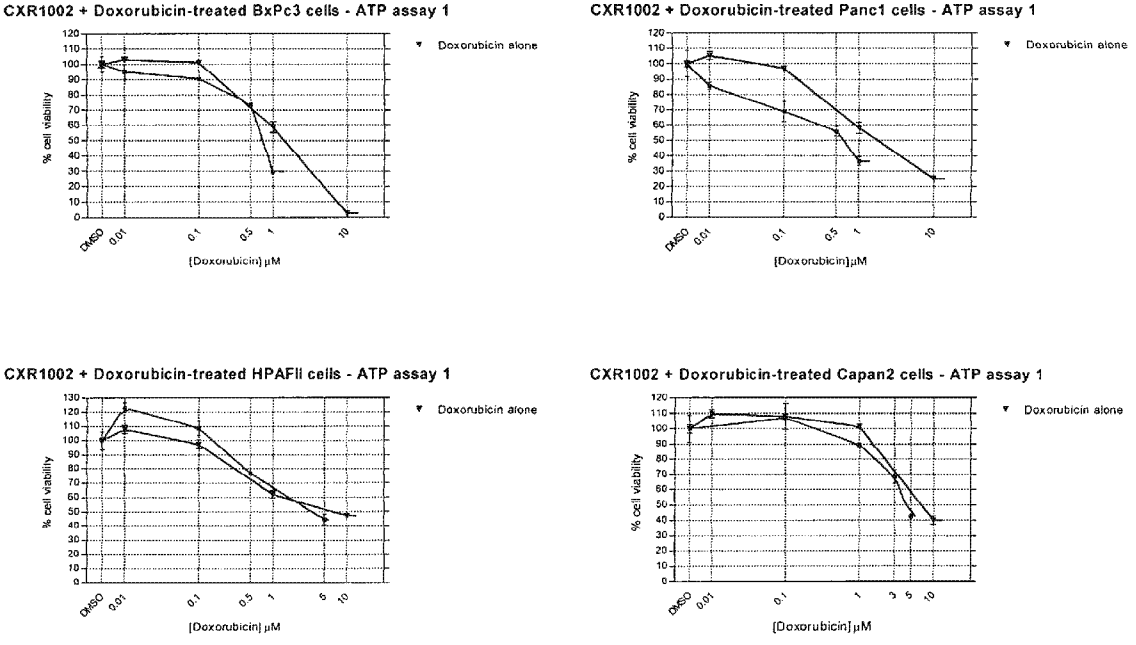


Figure 30

Ovarian Cell Lines treated with CXR 1002 & Doxorubicin

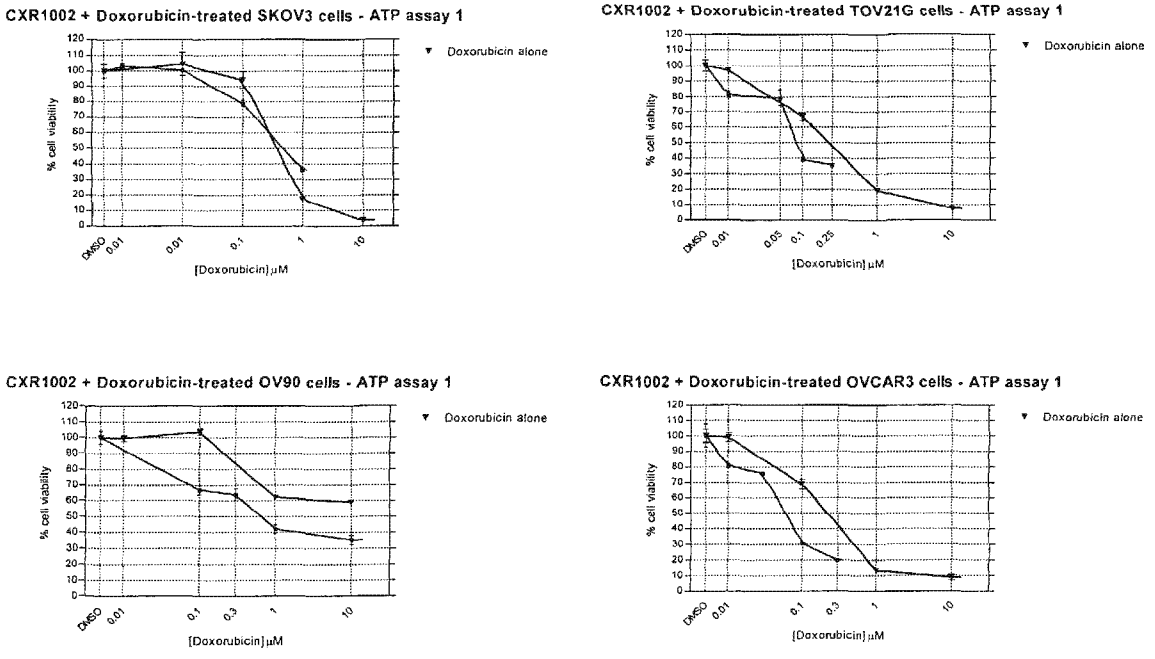


Figure 31

Sarcoma Cell Lines treated with CXR 1002 & Doxorubicin

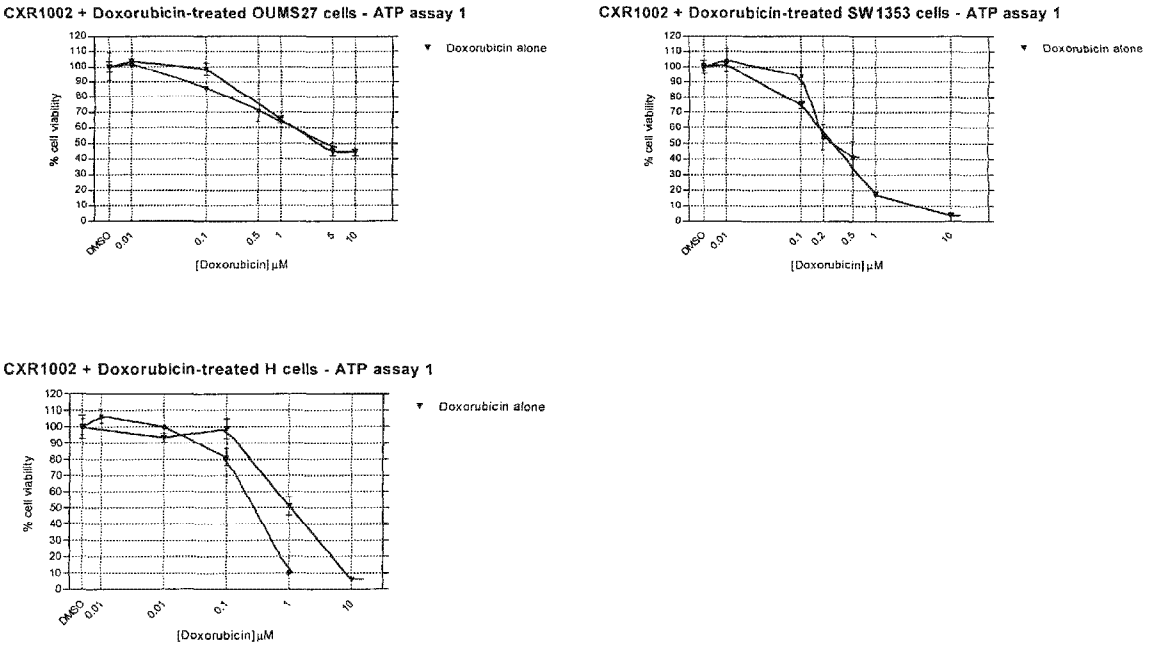
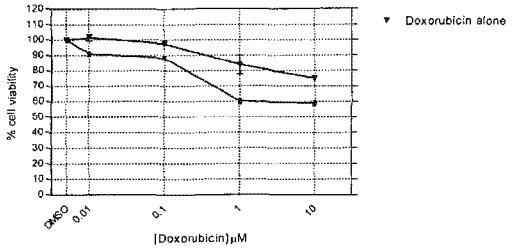
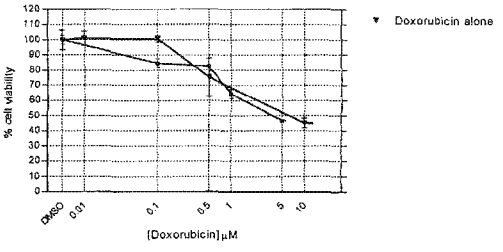


Figure 32
Miscellaneous Cell Lines treated with CXR 1002 & Doxorubicin

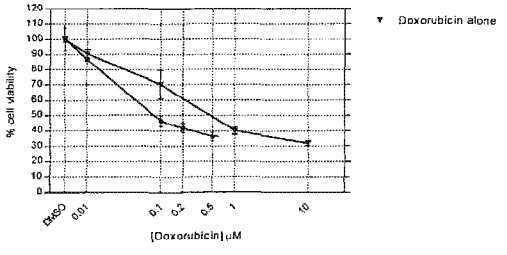
CXR1002 + Doxorubicin-treated PC3 cells - ATP assay 1



CXR1002 + Doxorubicin-treated CACO2 cells - ATP assay 1



CXR1002 + Doxorubicin-treated HepG2 cells - ATP assay 1



CXR1002 + Doxorubicin-treated MDA-MB-157 cells - ATP assay 1

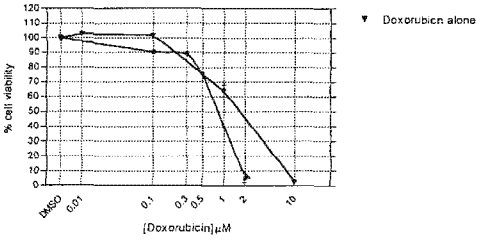
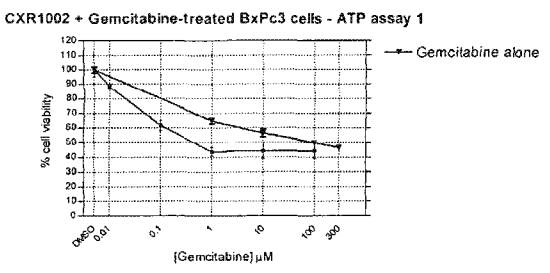
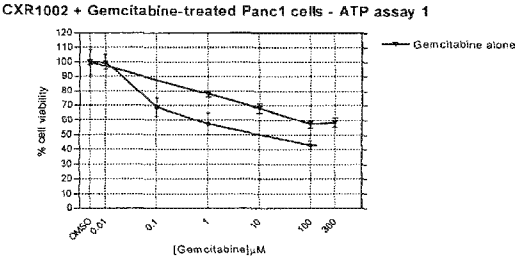
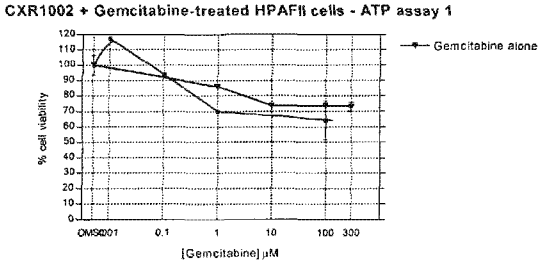


Figure 33

Pancreatic Cell Lines treated with CXR 1002 & Gemcitabine







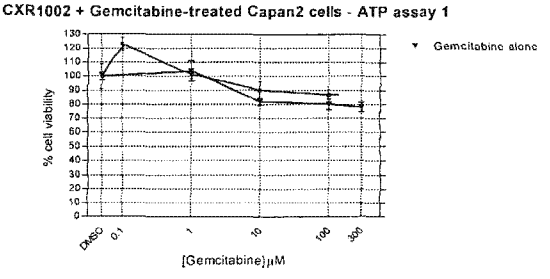
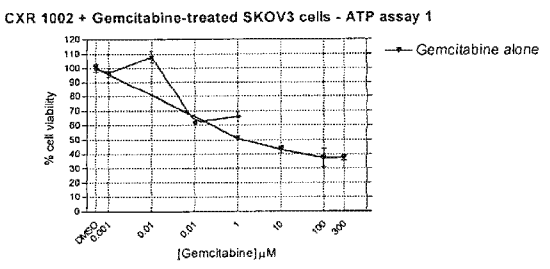
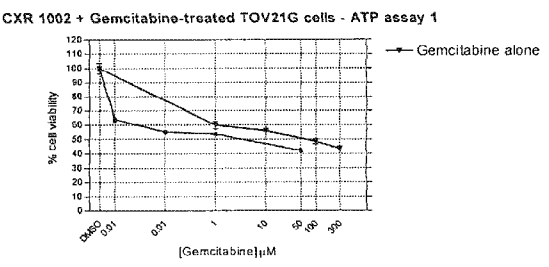
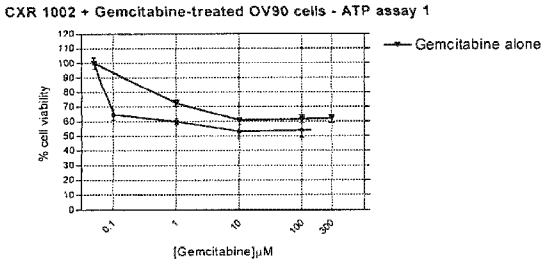


Figure 34

Ovarian Cell Lines treated with CXR 1002 & Gemcitabine







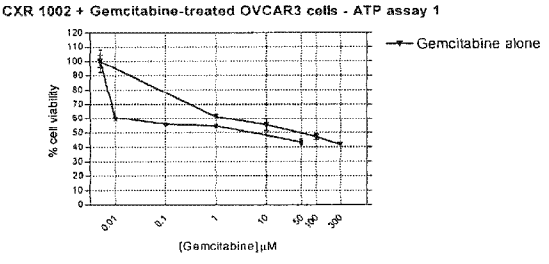


Figure 35

Sarcoma Cell Lines treated with CXR 1002 & Gemcitabine

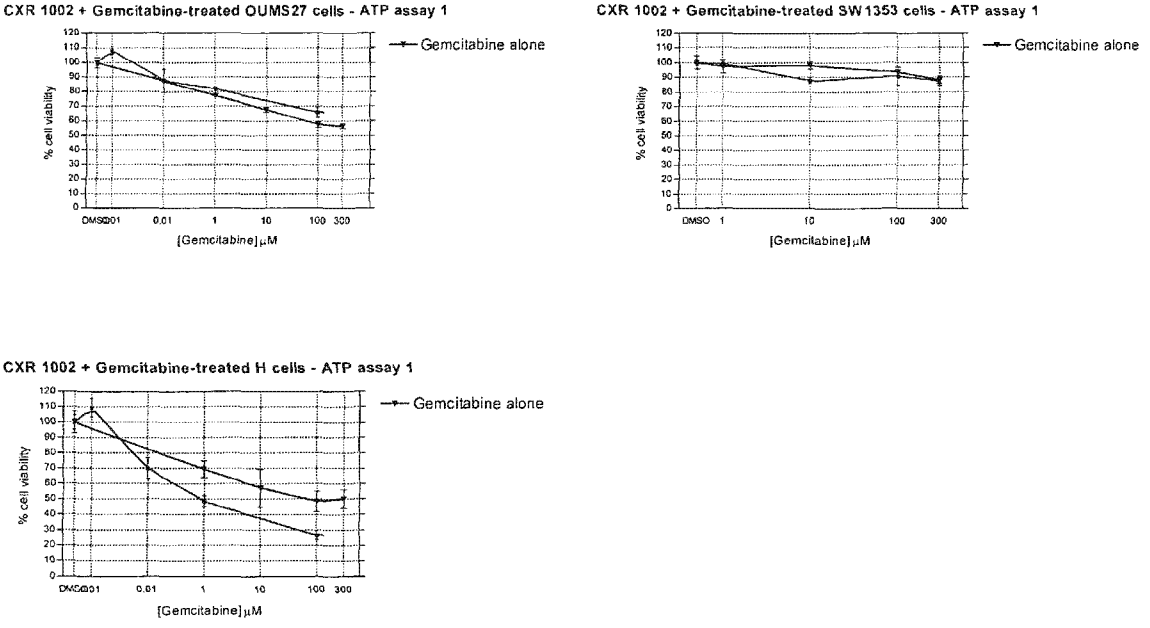


Figure 36

Miscellaneous Cell Lines treated with CXR 1002 & Gemcitabine

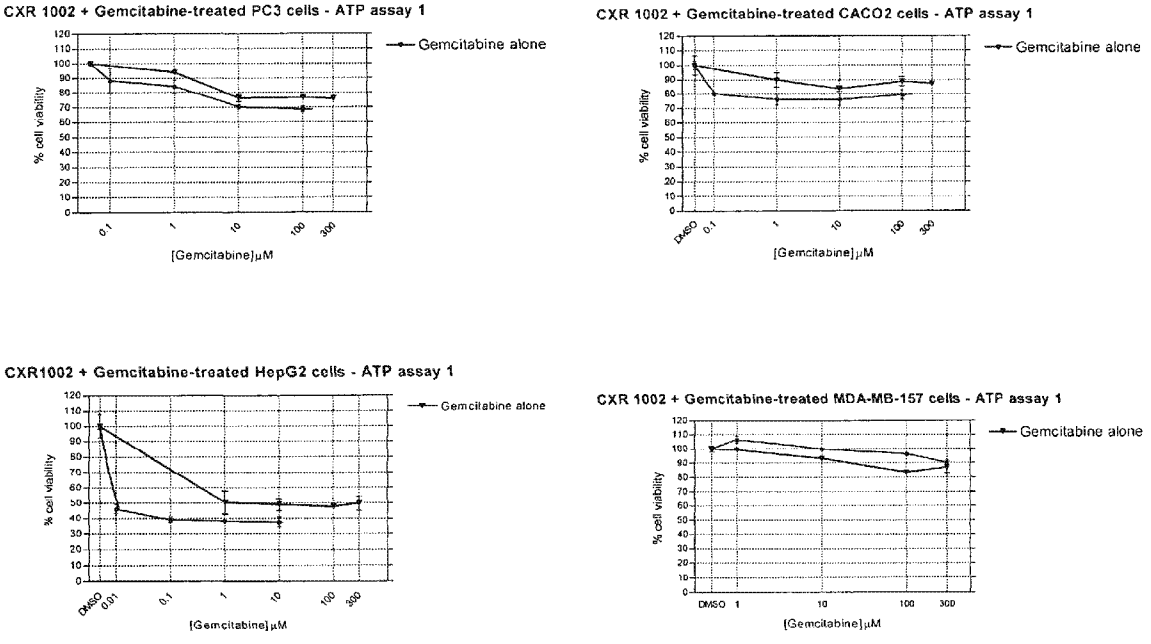
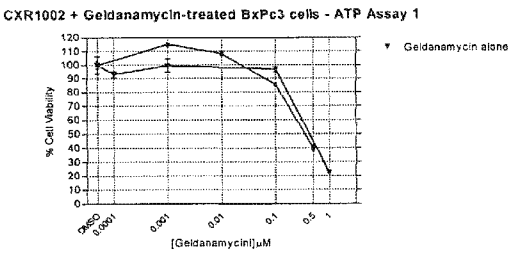
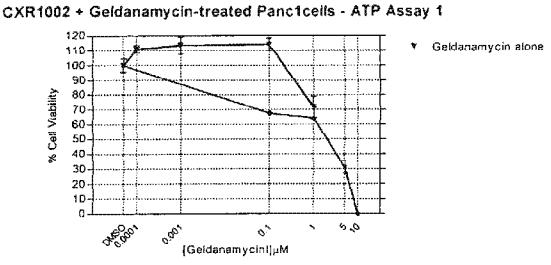
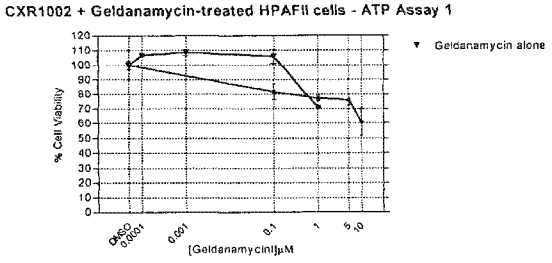


Figure 37

Pancreatic Cell Lines treated with CXR1002 & Geldanamycin







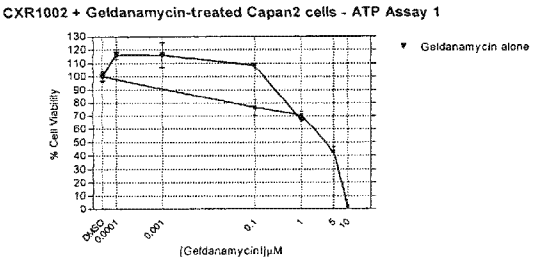


Figure 38

Ovarian Cell Lines treated with CXR1002 & Geldanamycin

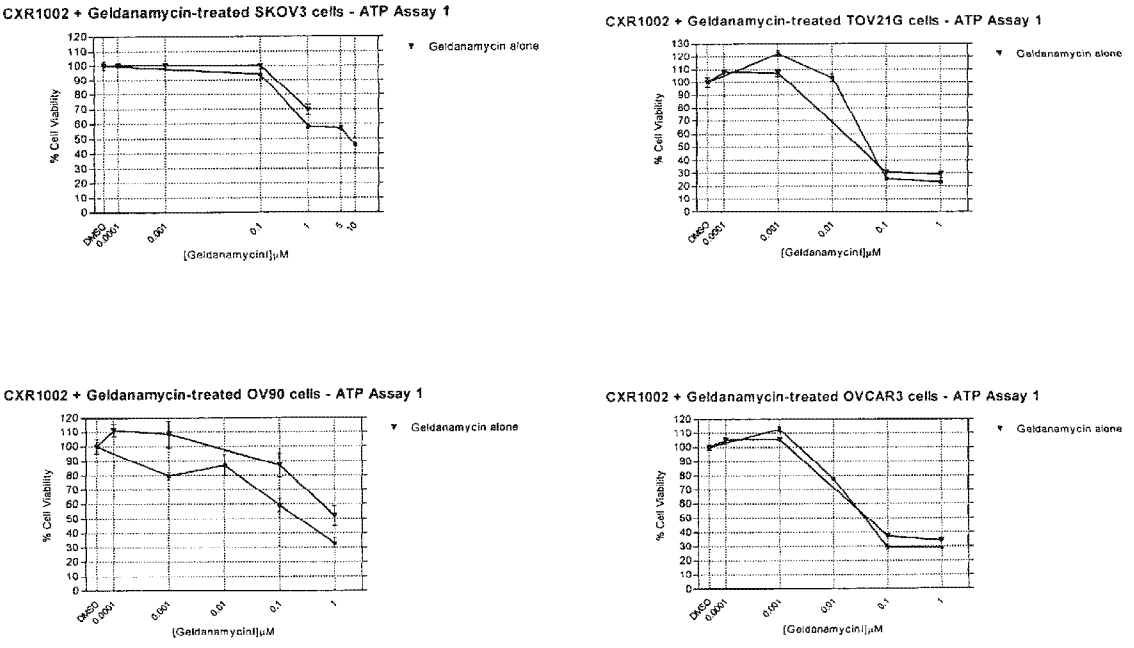
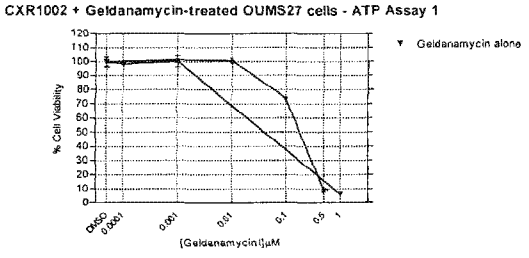
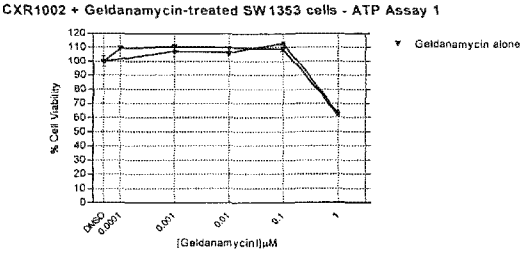


Figure 39

Sarcoma Cell Lines treated with CXR1002 & Geldanamycin





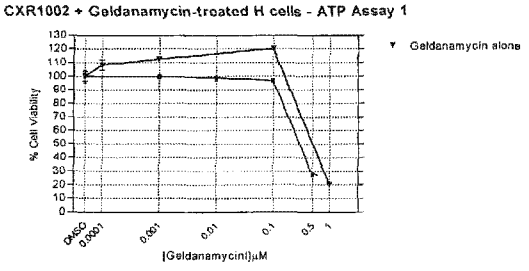
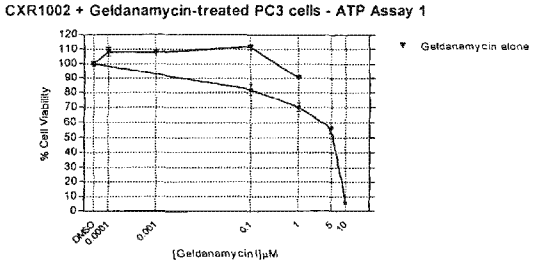
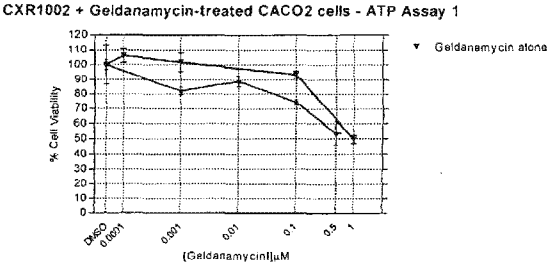
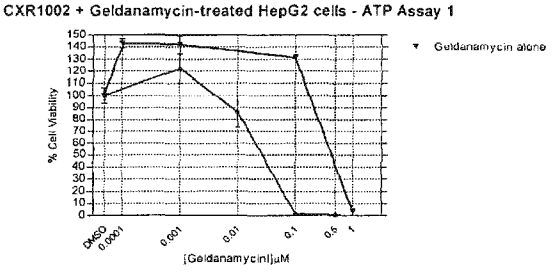


Figure 40

Miscellaneous Cell Lines treated with CXR1002 & Geldanamycin







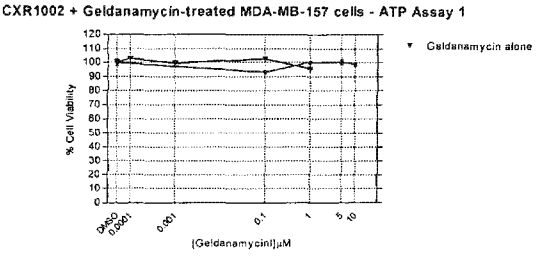


Figure 41

Pancreatic Cell Lines treated with CXR1002 & 5FU

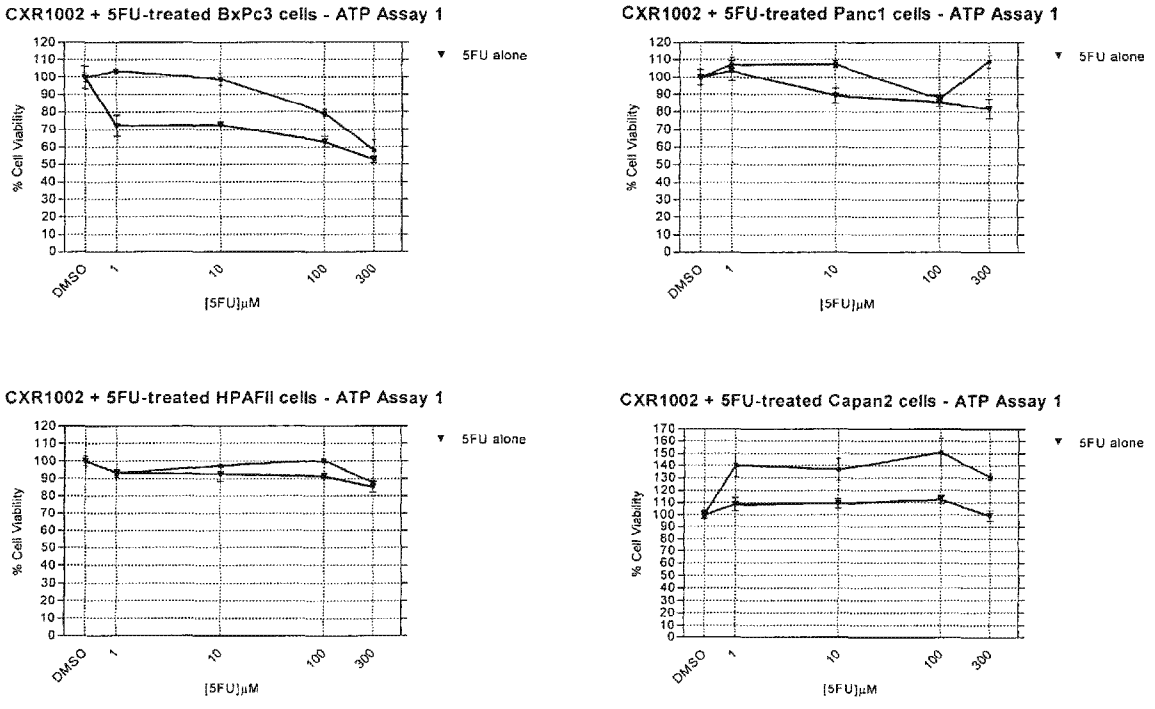


Figure 42

Ovarian Cell Lines treated with CXR1002 & 5FU

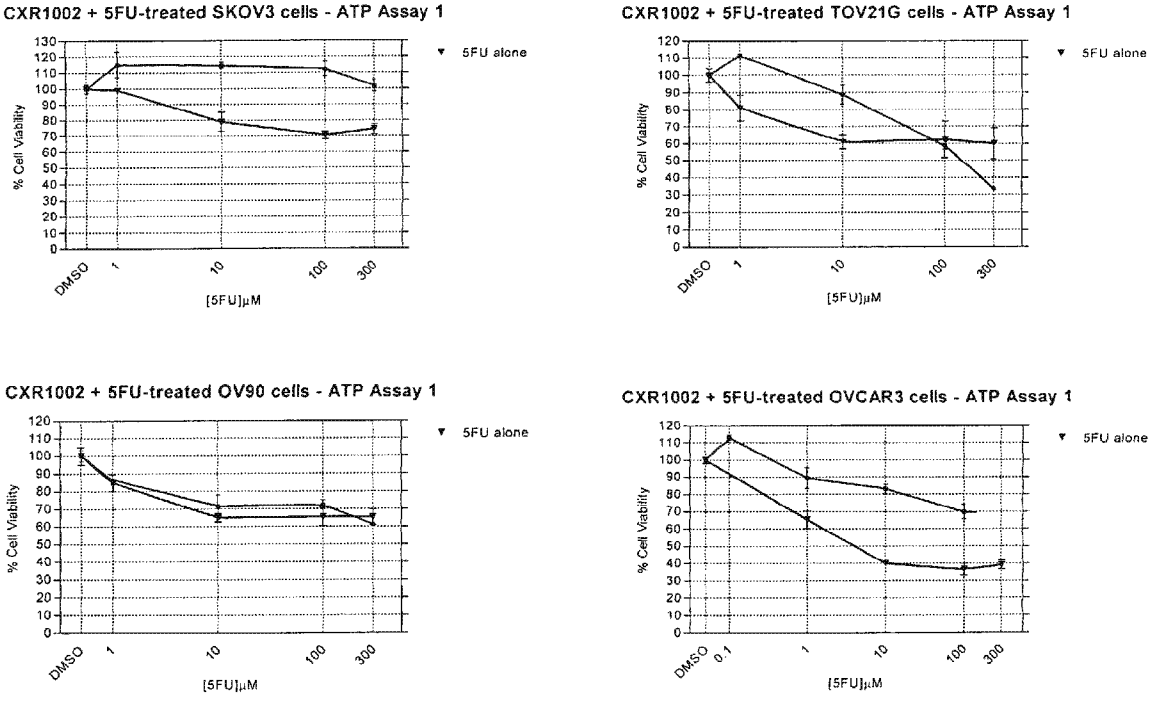


Figure 43

Sarcoma Cell Lines treated with CXR1002 & 5FU

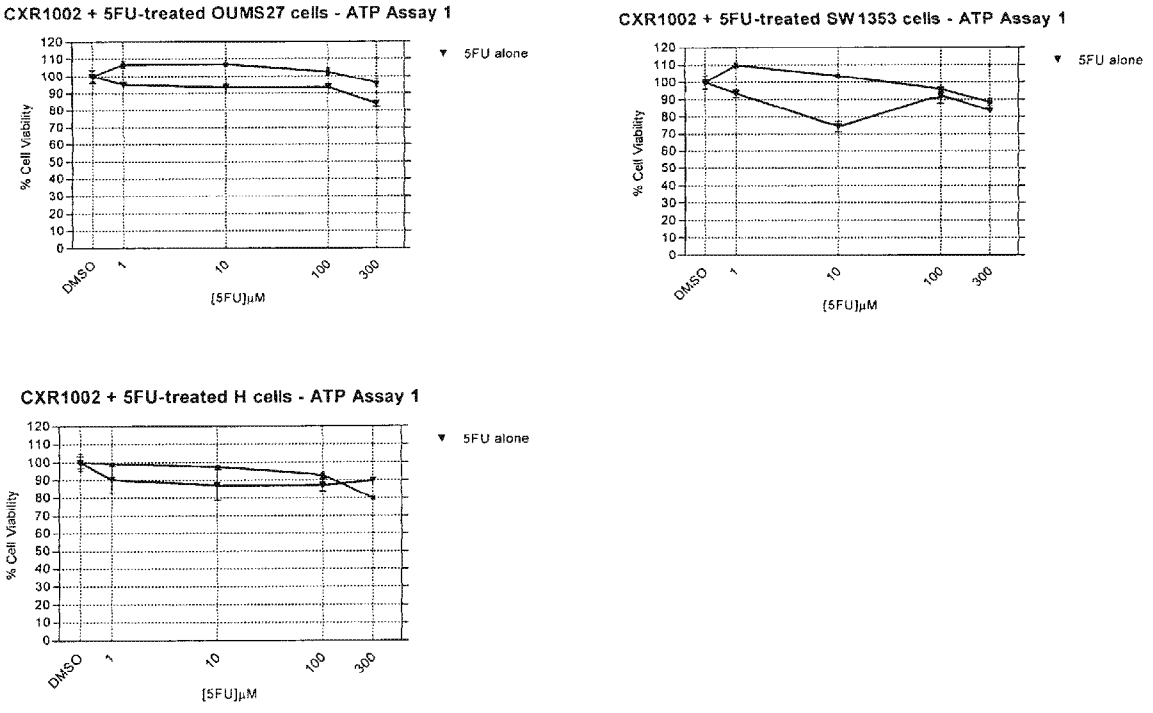


Figure 44

Miscellaneous Cell Lines treated with CXR1002 & 5FU

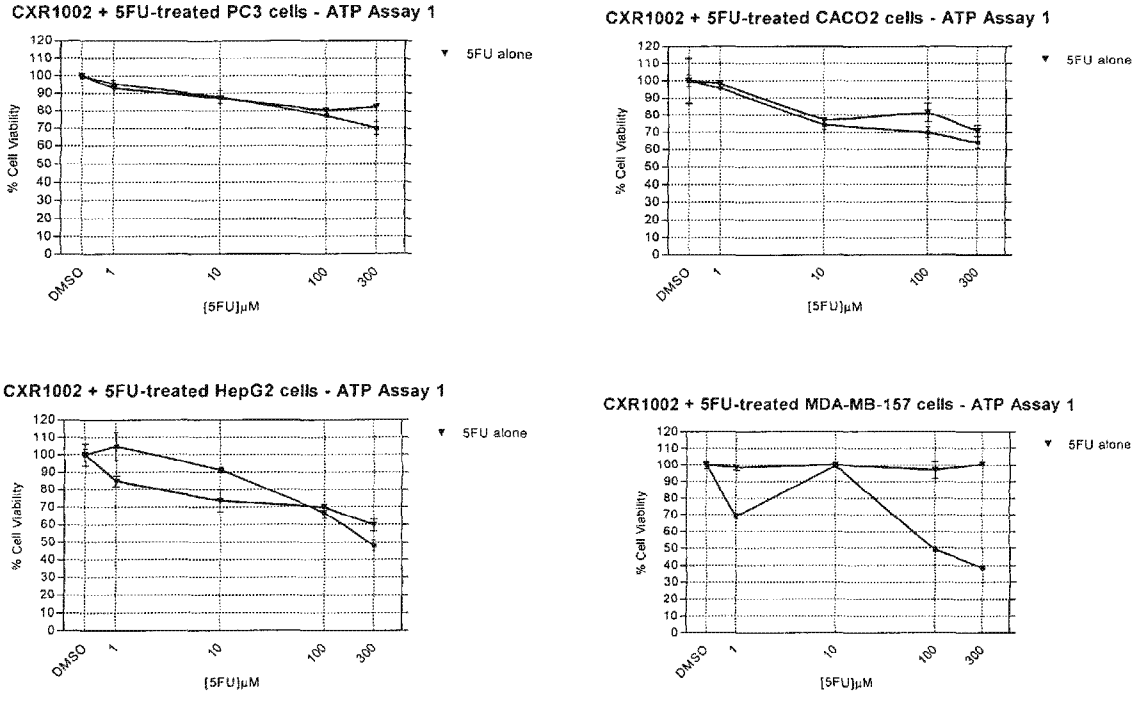
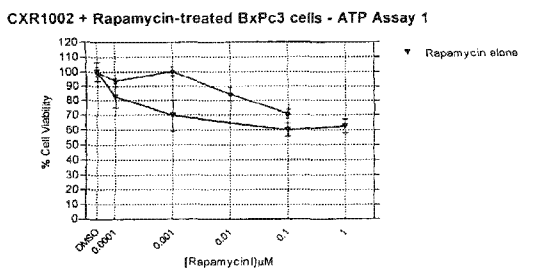
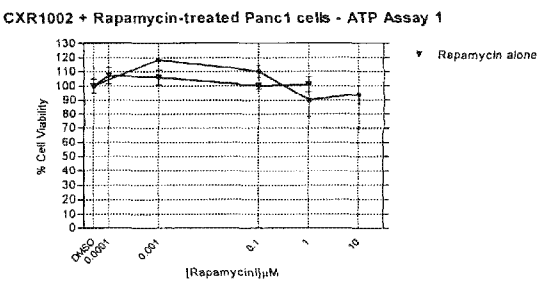
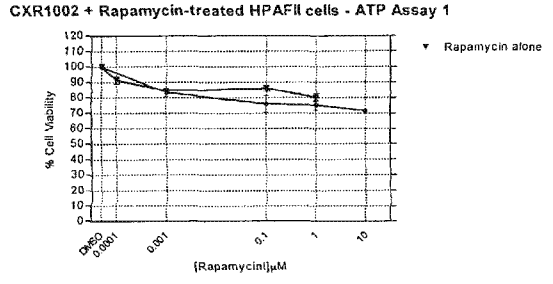


Figure 45

Pancreatic Cell Lines treated with CXR1002 & Rapamycin







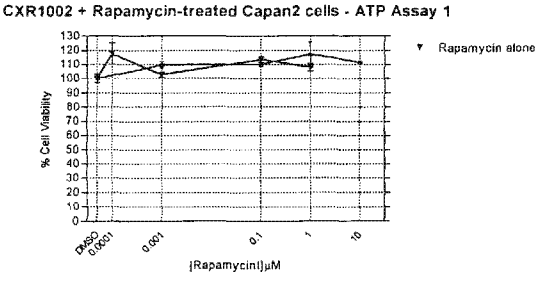


Figure 46

Ovarian Cell Lines treated with CXR1002 & Rapamycin

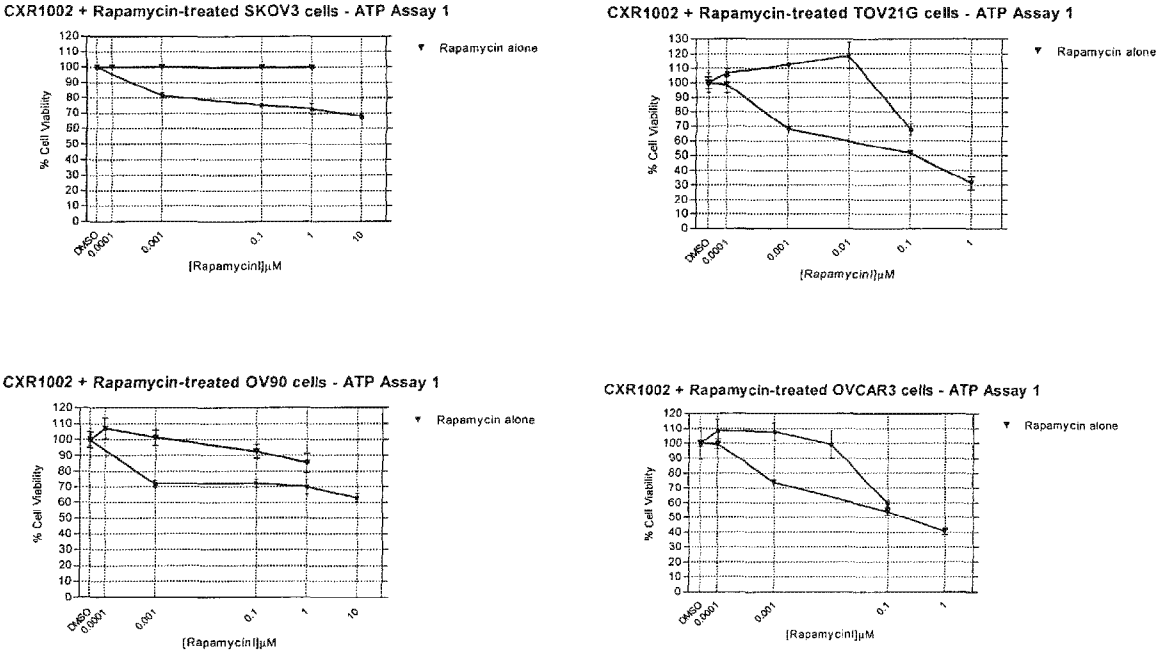


Figure 47

Sarcoma Cell Lines treated with CXR1002 & Rapamycin

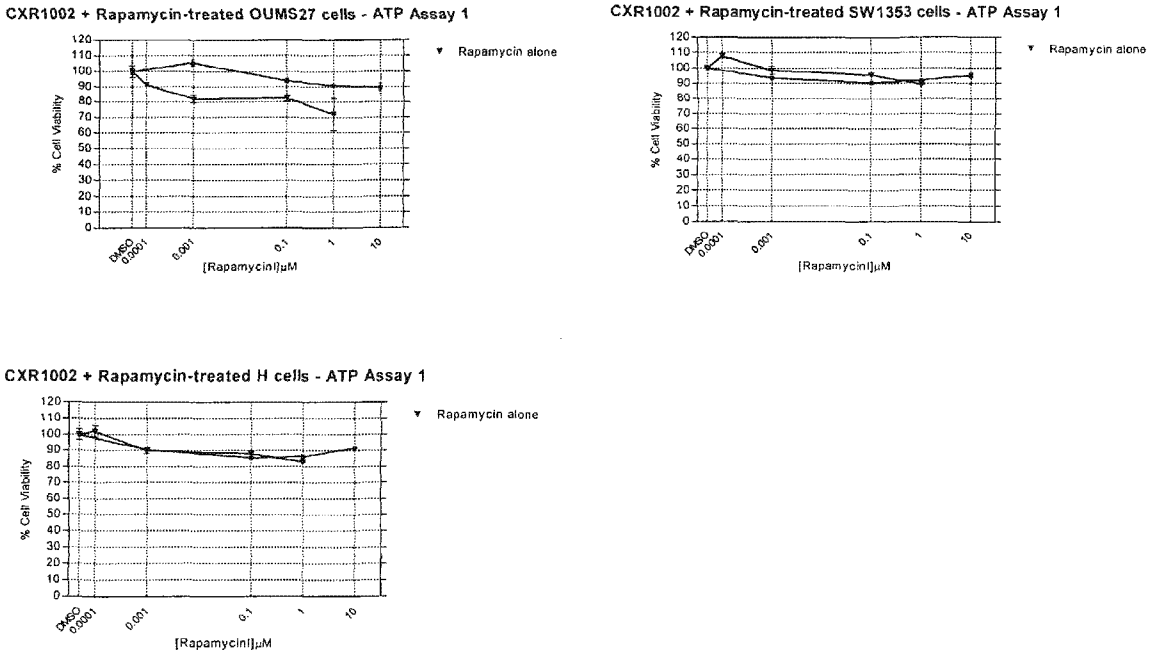


Figure 48

Miscellaneous Cell Lines treated with CXR1002 & Rapamycin

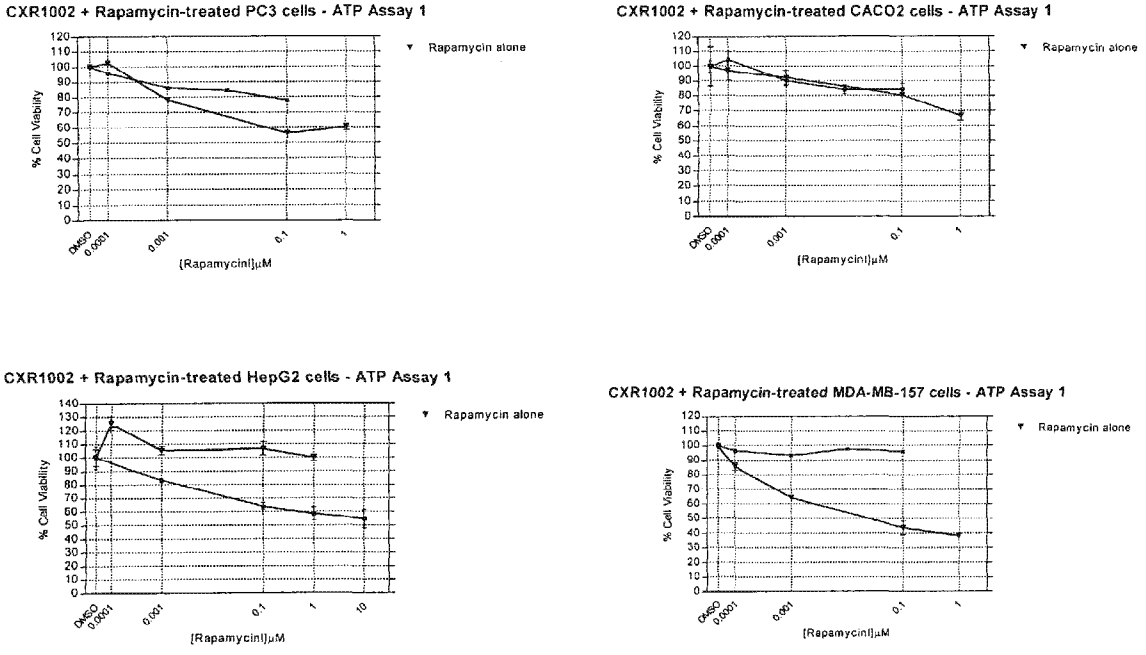


Figure 49

Pancreatic Cell Lines treated with CXR1002 & Roscovitine

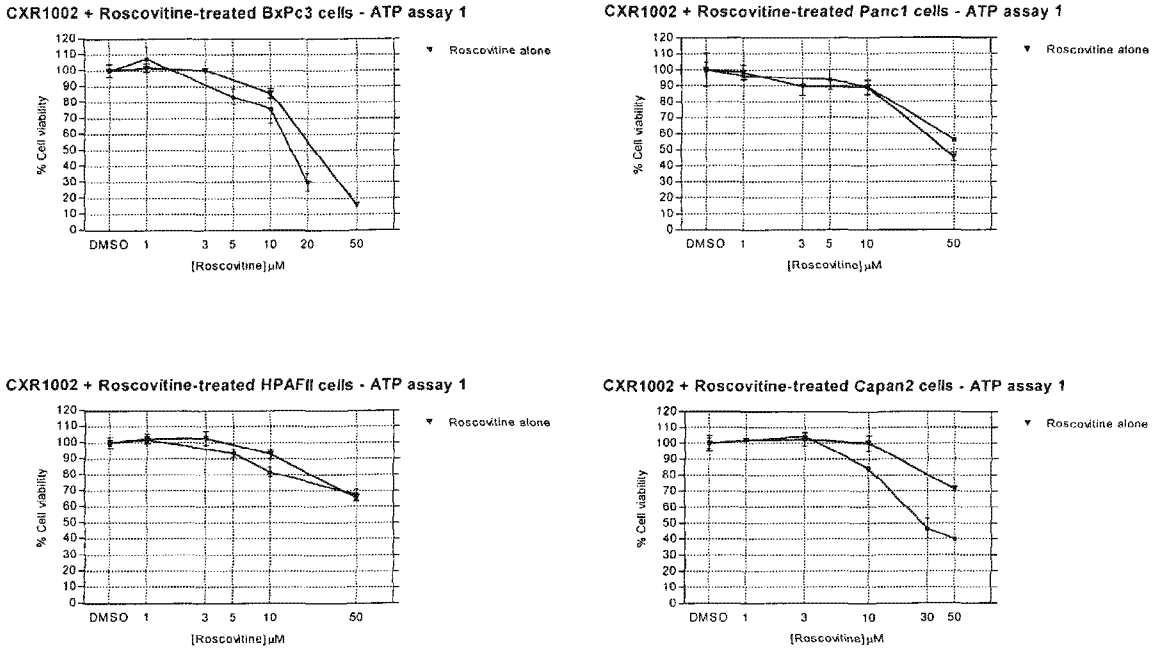


Figure 50

Ovarian Cell Lines treated with CXR1002 & Roscovitine

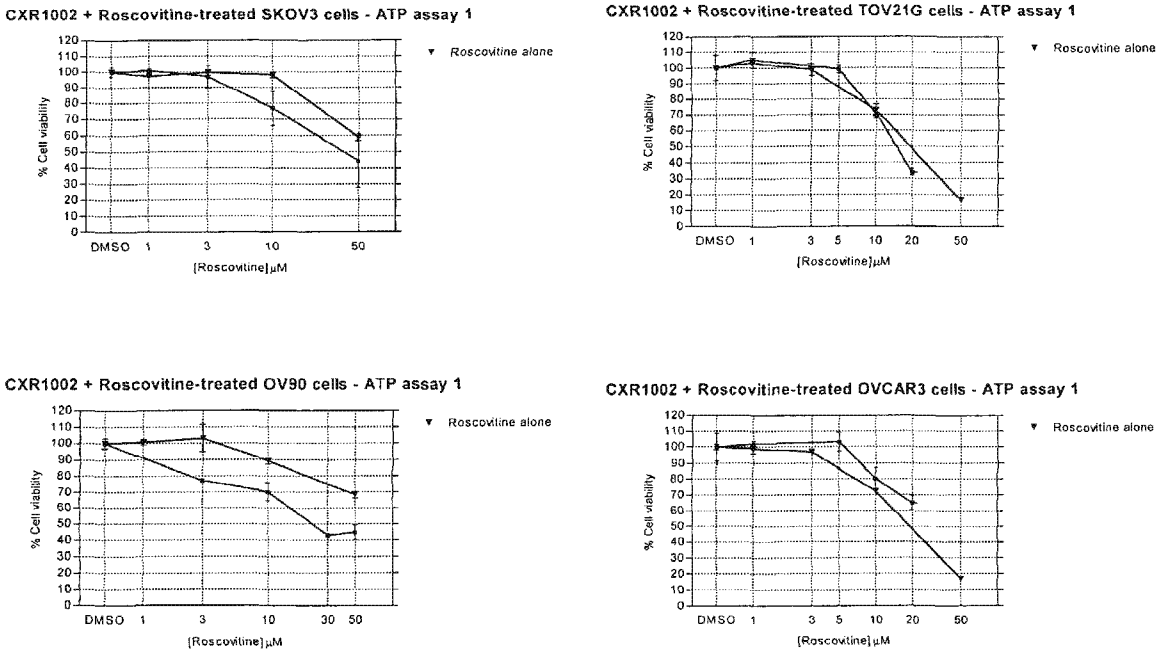
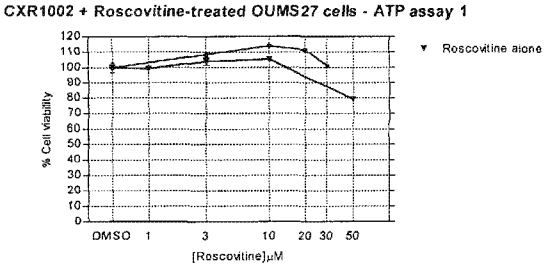
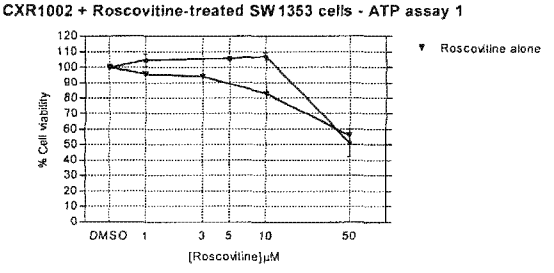


Figure 51

Sarcoma Cell Lines treated with CXR 1002 & Roscovitine





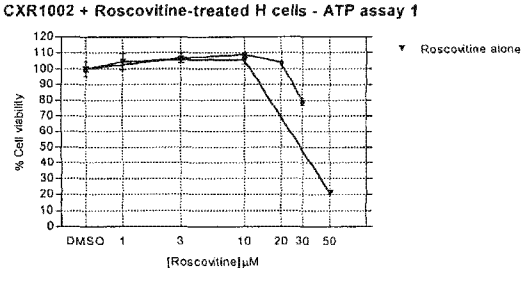


Figure 52

Miscellaneous Cell Lines treated with CXR1002 & Roscovitine

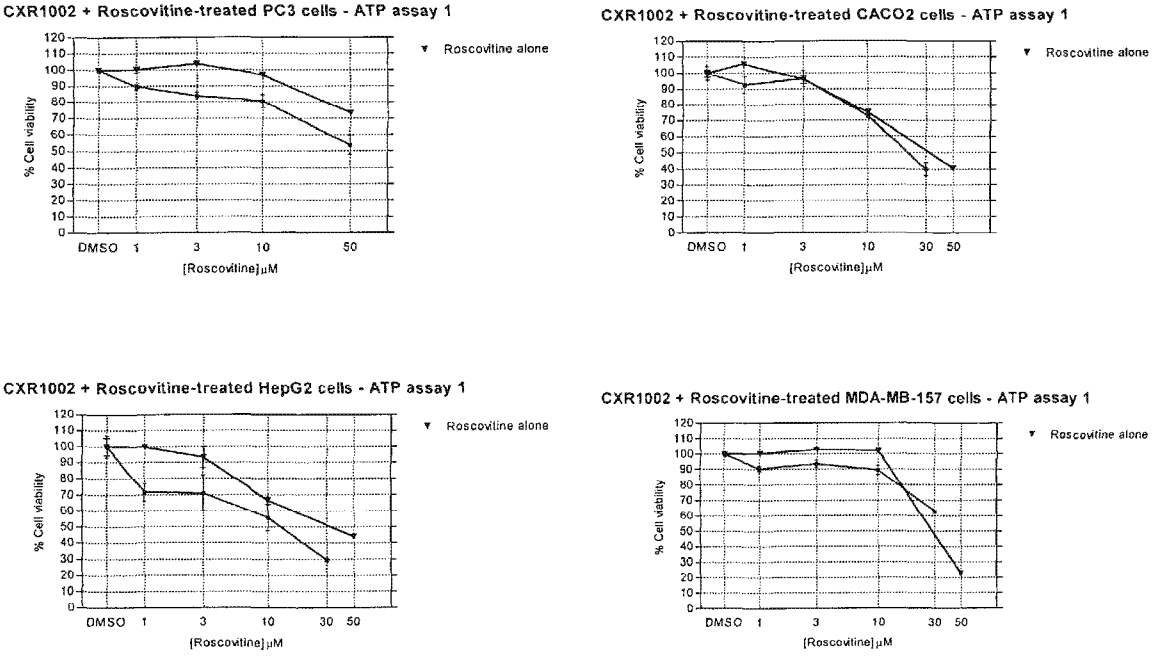


Figure 53

Pancreatic Cell Lines treated with CXR1002 & Docetaxel

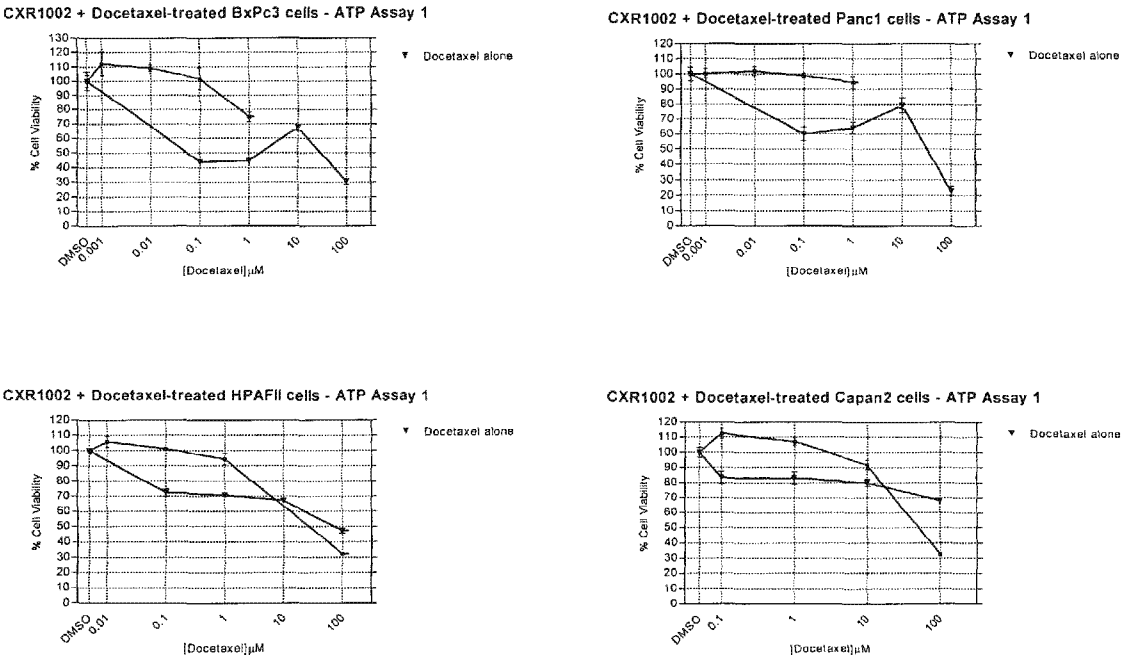
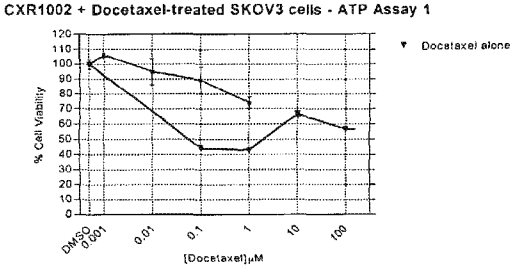
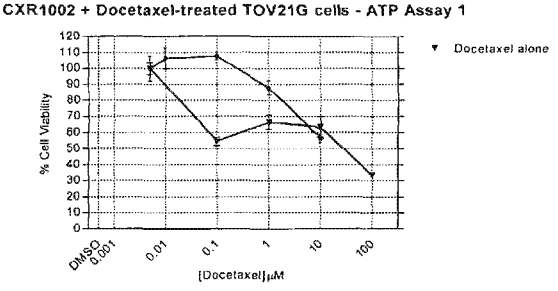
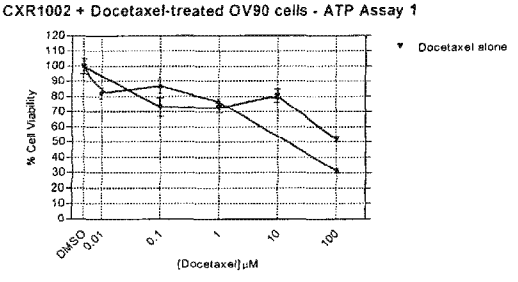


Figure 54

Ovarian Cell Lines treated with CXR1002 & Docetaxel







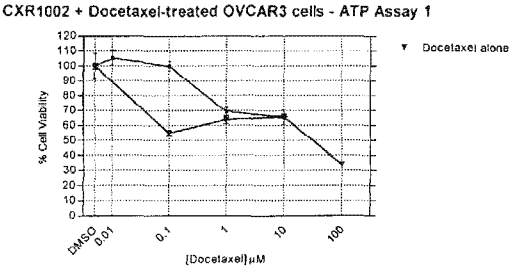
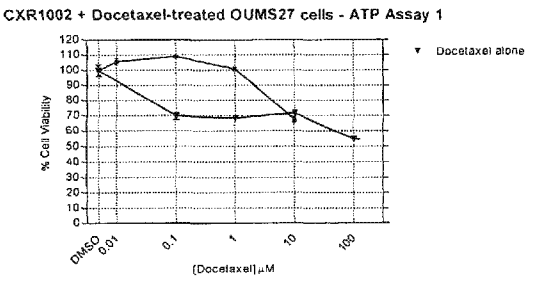
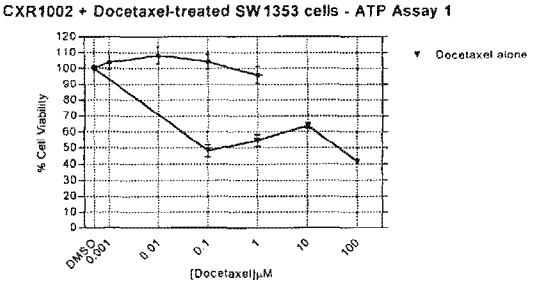


Figure 55

Sarcoma Cell Lines treated with CXR1002 & Docetaxel





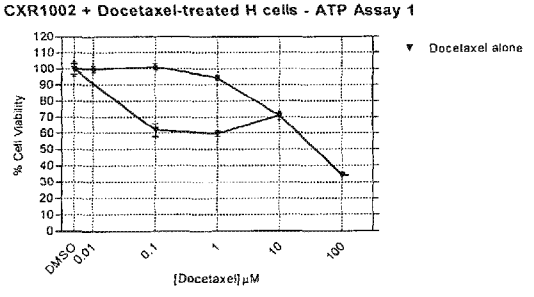
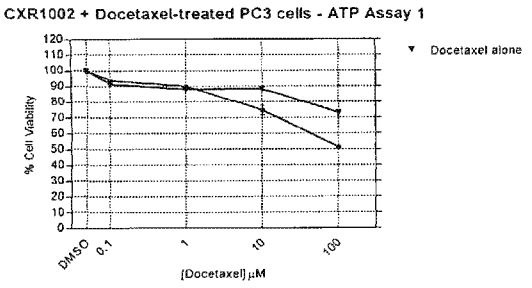
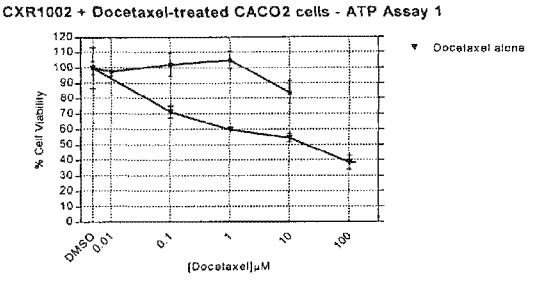
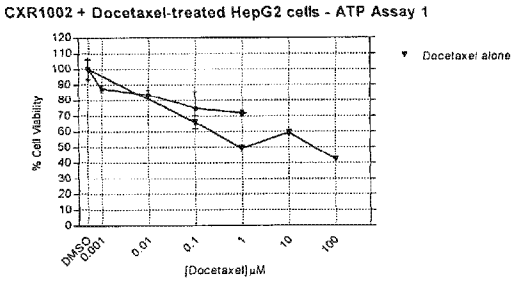


Figure 56

Miscellaneous Cell Lines treated with CXR1002 & Docetaxel







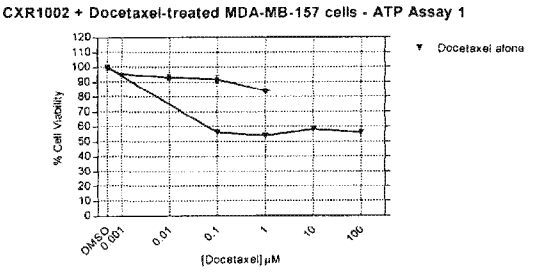


Figure 57

Pancreatic Cell Lines treated with CXR 1002 & Cisplatin

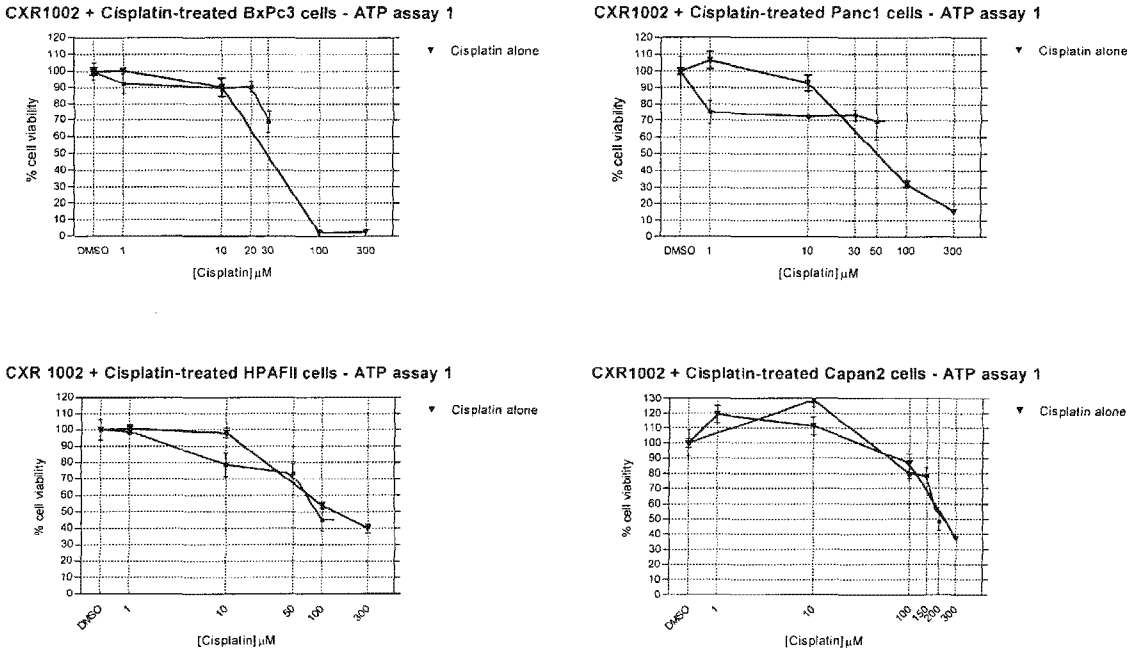


Figure 58

Ovarian Cell Lines treated with CXR 1002 & Cisplatin

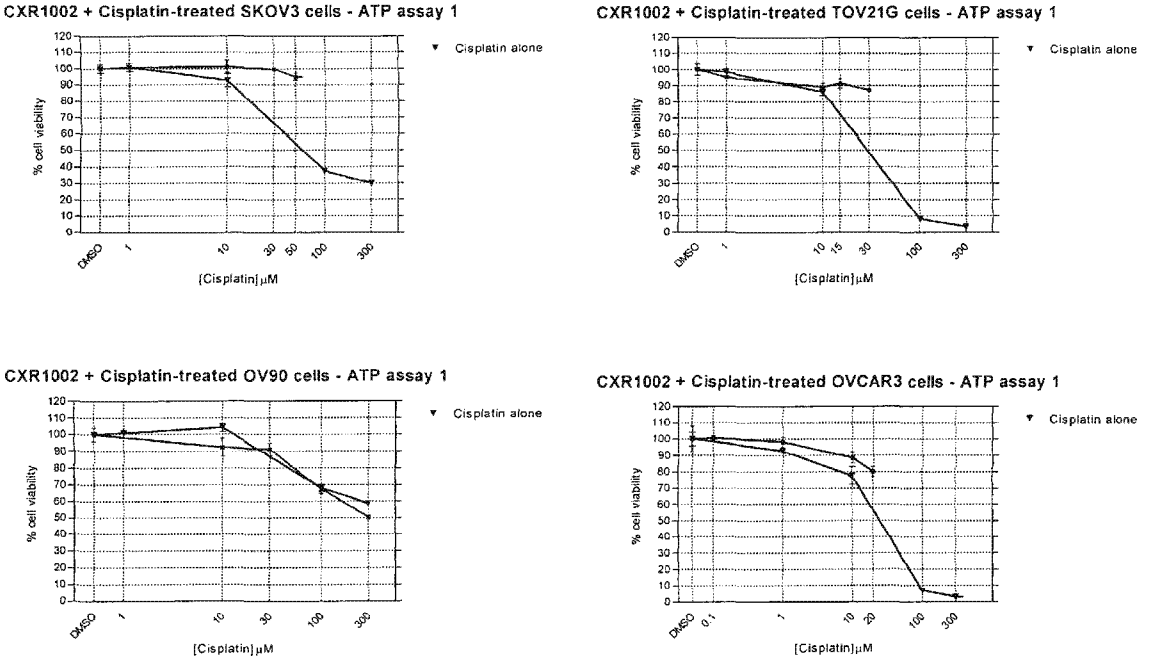
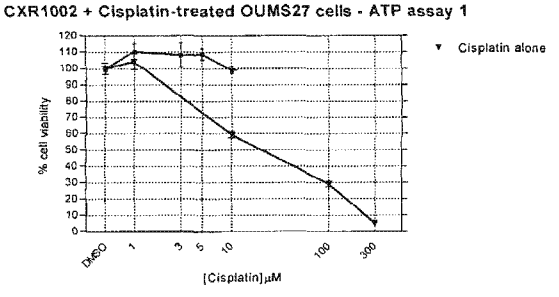
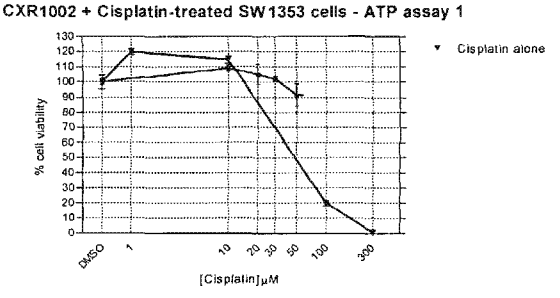


Figure 59

Sarcoma Cell Lines treated with CXR 1002 & Cisplatin





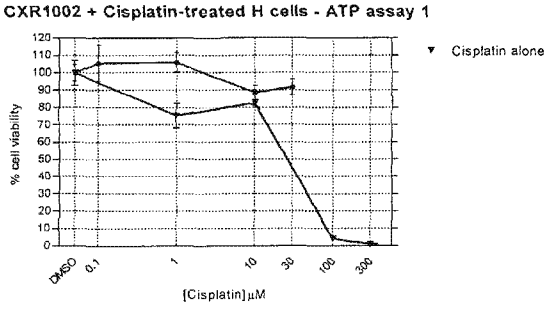


Figure 60

Miscellaneous Cell Lines treated with CXR 1002 & Cisplatin

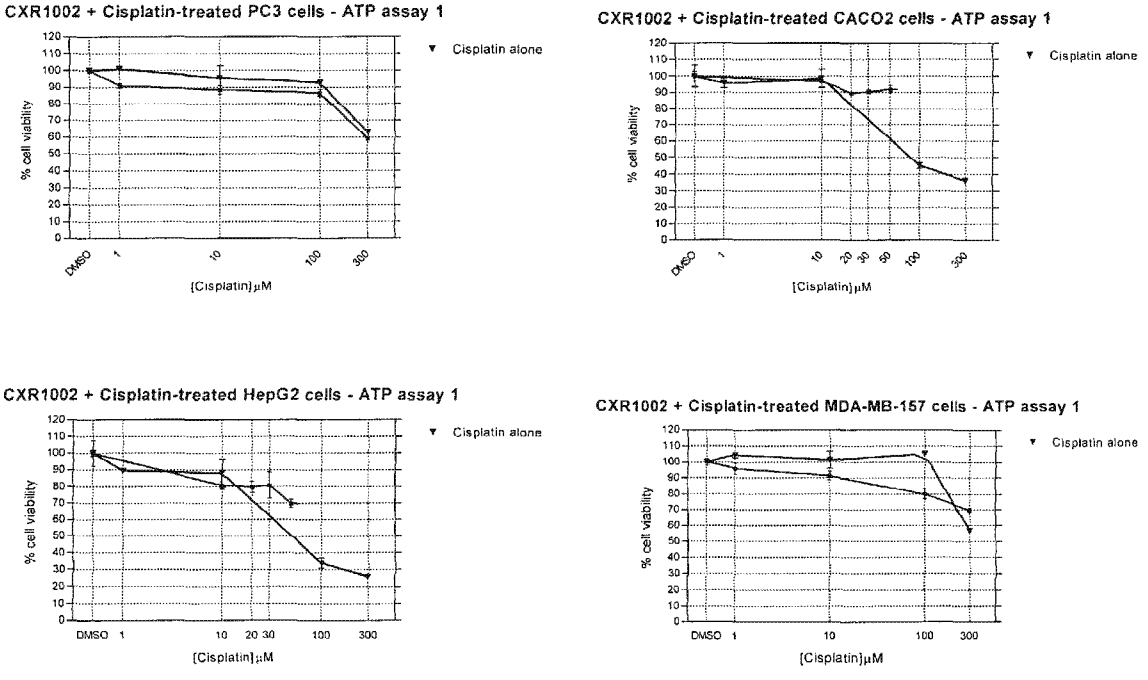


Figure 61

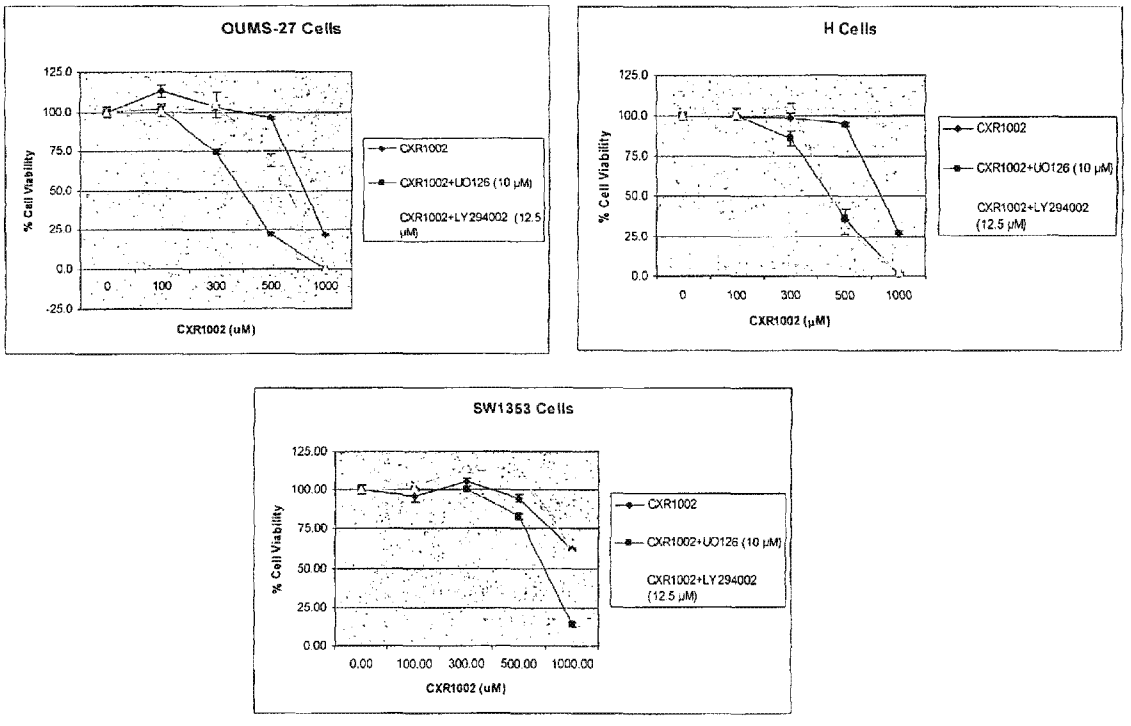

OUMS-27 Cells
% Cell Viability
125.0
100.0
75.0
50.0
25.0
0.0
-25.0
0
100
300
500
1000
CXR1002 (uM)
CXR1002
CXR1002+UO126 (10 μM)
CXR1002+LY294002 (12.5 μM)
H Cells
% Cell Viability
125.0
100.0
75.0
50.0
25.0
0.0
0
100
300
500
1000
CXR1002 (μM)
CXR1002
CXR1002+UO126 (10 μM)
CXR1002+LY294002 (12.5 μM)
SW1353 Cells
% Cell Viability
125.00
100.00
75.00
50.00
25.00
0.00
0.00
100.00
300.00
500.00
1000.00
CXR1002 (uM)
CXR1002
CXR1002+UO126 (10 μM)
CXR1002+LY294002 (12.5 μM)

Figure 62

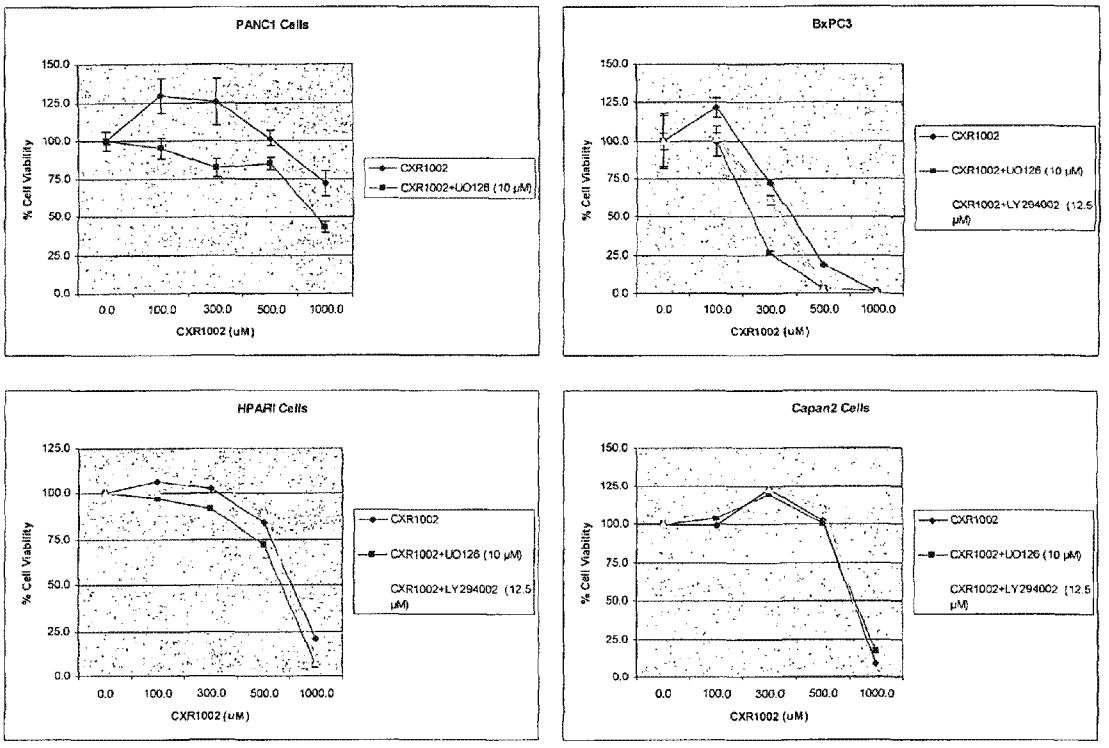

PANC1 Cells
% Cell Viability
150.0
125.0
100.0
75.0
50.0
25.0
0.0
0.0
100.0
300.0
500.0
1000.0
CXR1002 (uM)
CXR1002
CXR1002+UO126 (10 μM)
BxPC3
% Cell Viability
CXR1002 (uM)
CXR1002
CXR1002+UO126 (10 μM)
CXR1002+LY294002 (12.5 μM)
HPARI Cells
% Cell Viability
125.0
100.0
75.0
50.0
25.0
0.0
CXR1002 (uM)
CXR1002
CXR1002+UO126 (10 μM)
CXR1002+LY294002 (12.5 μM)
Capan2 Cells
% Cell Viability
CXR1002 (uM)
CXR1002
CXR1002+UO126 (10 μM)
CXR1002+LY294002 (12.5 μM)

Figure 63

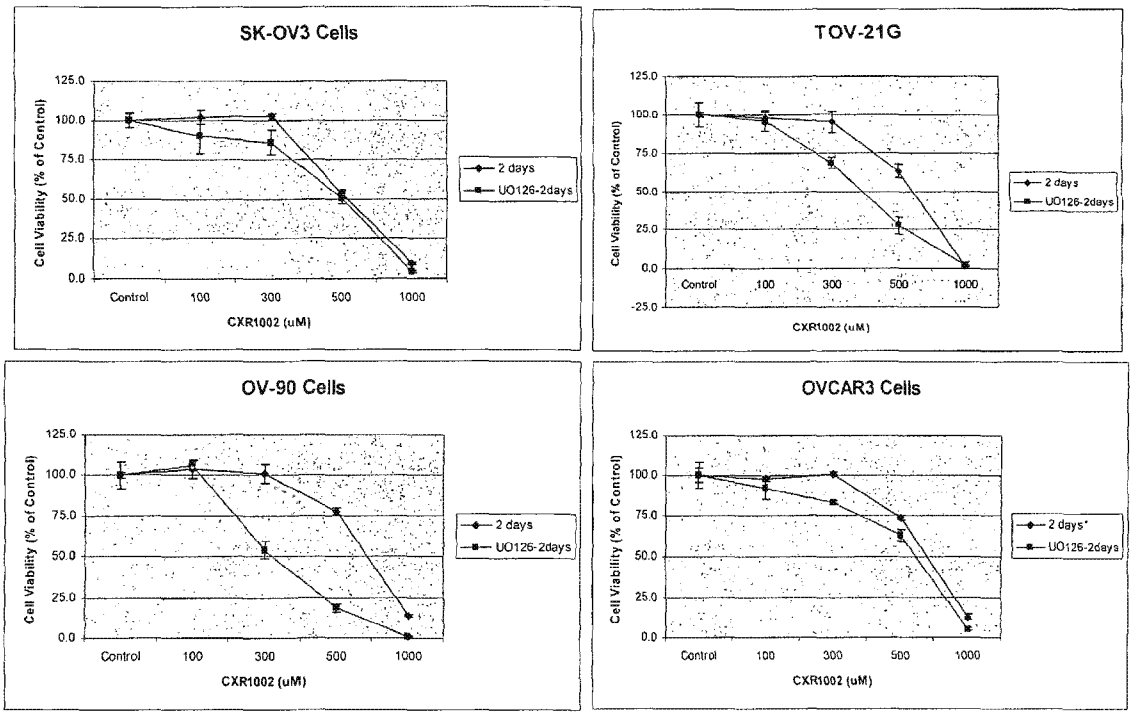

SK-OV3 Cells
Cell Viability (% of Control)
125.0
100.0
75.0
50.0
25.0
0.0
Control
100
300
500
1000
CXR1002 (uM)
2 days
UO126-2days
TOV-21G
Cell Viability (% of Control)
125.0
100.0
75.0
50.0
25.0
0.0
-25.0
Control
100
300
500
1000
CXR1002 (uM)
2 days
UO126-2days
OV-90 Cells
Cell Viability (% of Control)
125.0
100.0
75.0
50.0
25.0
0.0
Control
100
300
500
1000
CXR1002 (uM)
2 days
UO126-2days
OVCAR3 Cells
Cell Viability (% of Control)
125.0
100.0
75.0
50.0
25.0
0.0
Control
100
300
500
1000
CXR1002 (uM)
2 days*
UO126-2days

Figure 64

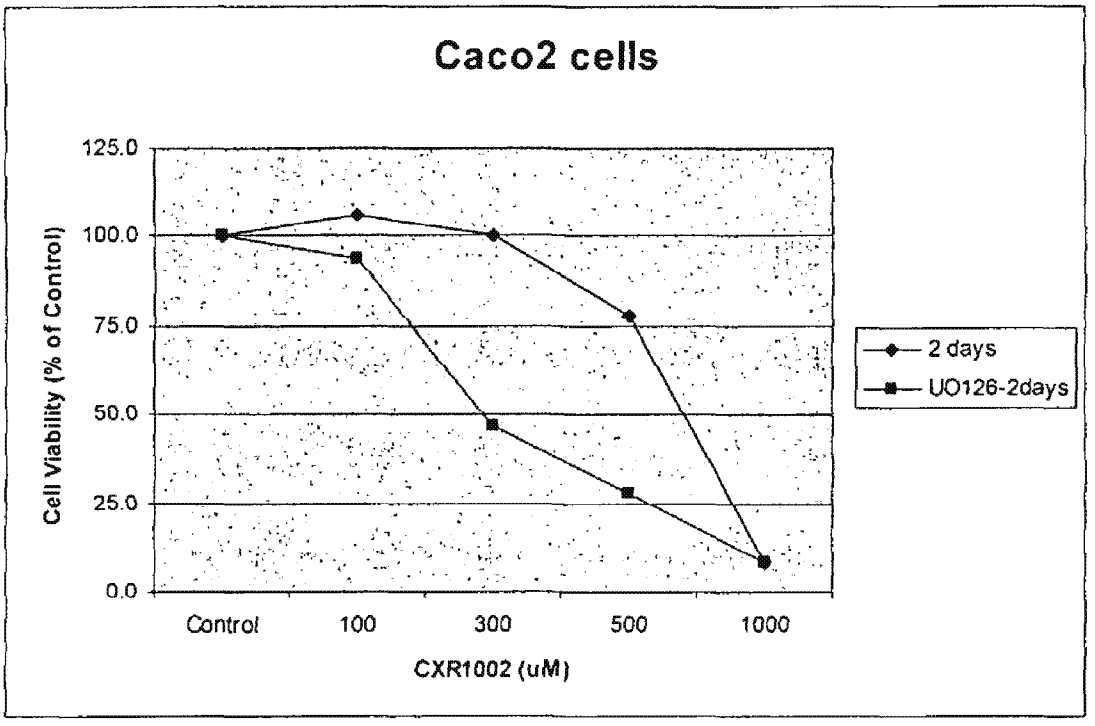

Caco2 cells
125.0
100.0
75.0
50.0
25.0
0.0
Cell Viability (% of Control)
Control
100
300
500
1000
CXR1002 (uM)
2 days
UO126-2days

Figure 65

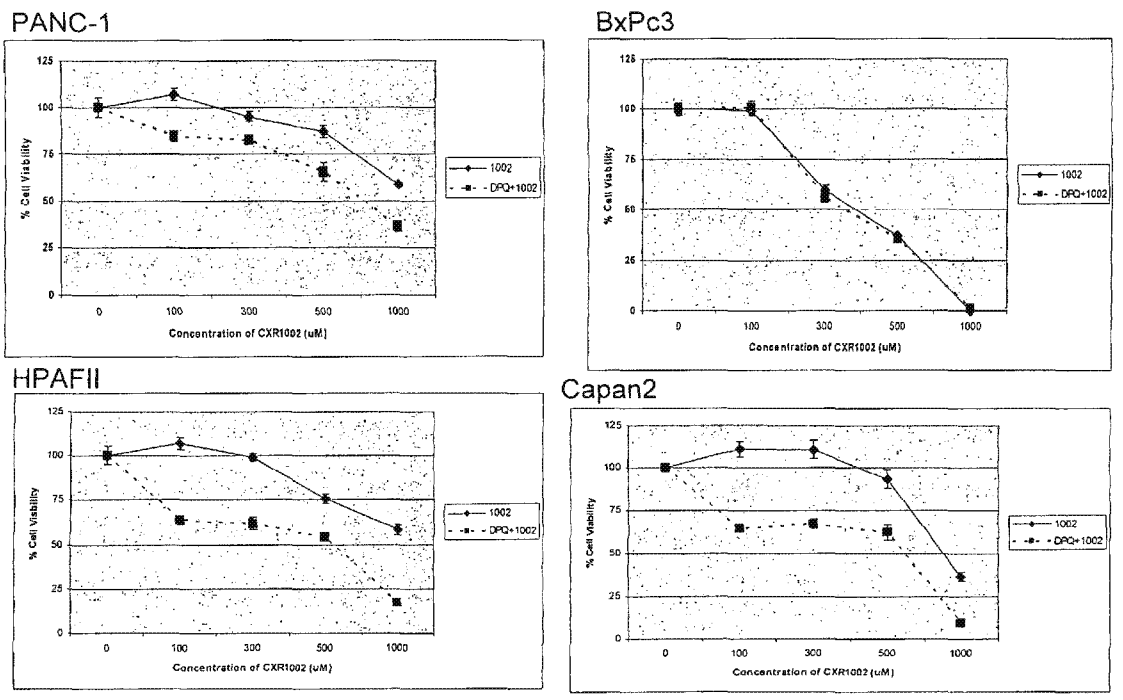

PANC-1
BxPc3
HPAFII
Capan2
% Cell Viability
Concentration of CXR1002 (uM)
0
100
300
500
1000
1002
DPQ+1002

Figure 66

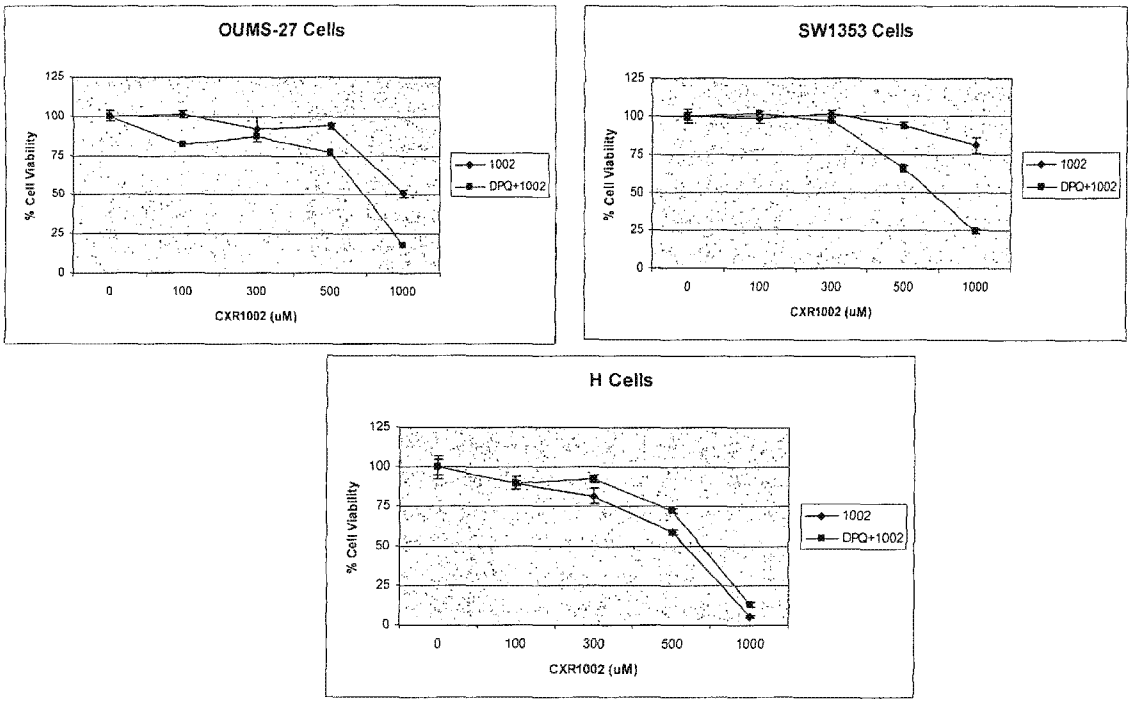

OUMS-27 Cells
% Cell Viability
125
100
75
50
25
0
0
100
300
500
1000
CXR1002 (uM)
1002
DPQ+1002
SW1353 Cells
% Cell Viability
125
100
75
50
25
0
0
100
300
500
1000
CXR1002 (uM)
1002
DPQ+1002
H Cells
% Cell Viability
125
100
75
50
25
0
0
100
300
500
1000
CXR1002 (uM)
1002
DPQ+1002

Figure 67

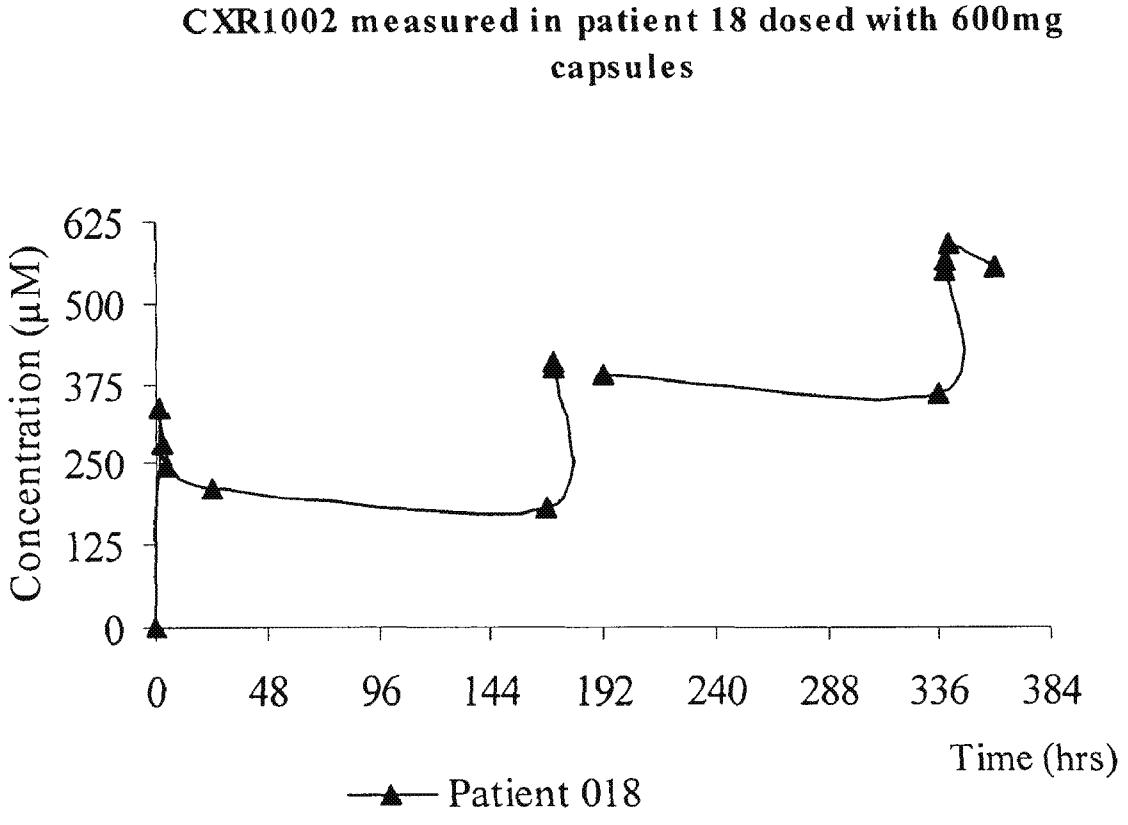

CXR1002 measured in patient 18 dosed with 600mg capsules
Concentration (µM)
625
500
375
250
125
0
0
48
96
144
192
240
288
336
384
Time (hrs)
Patient 018

Figure 68

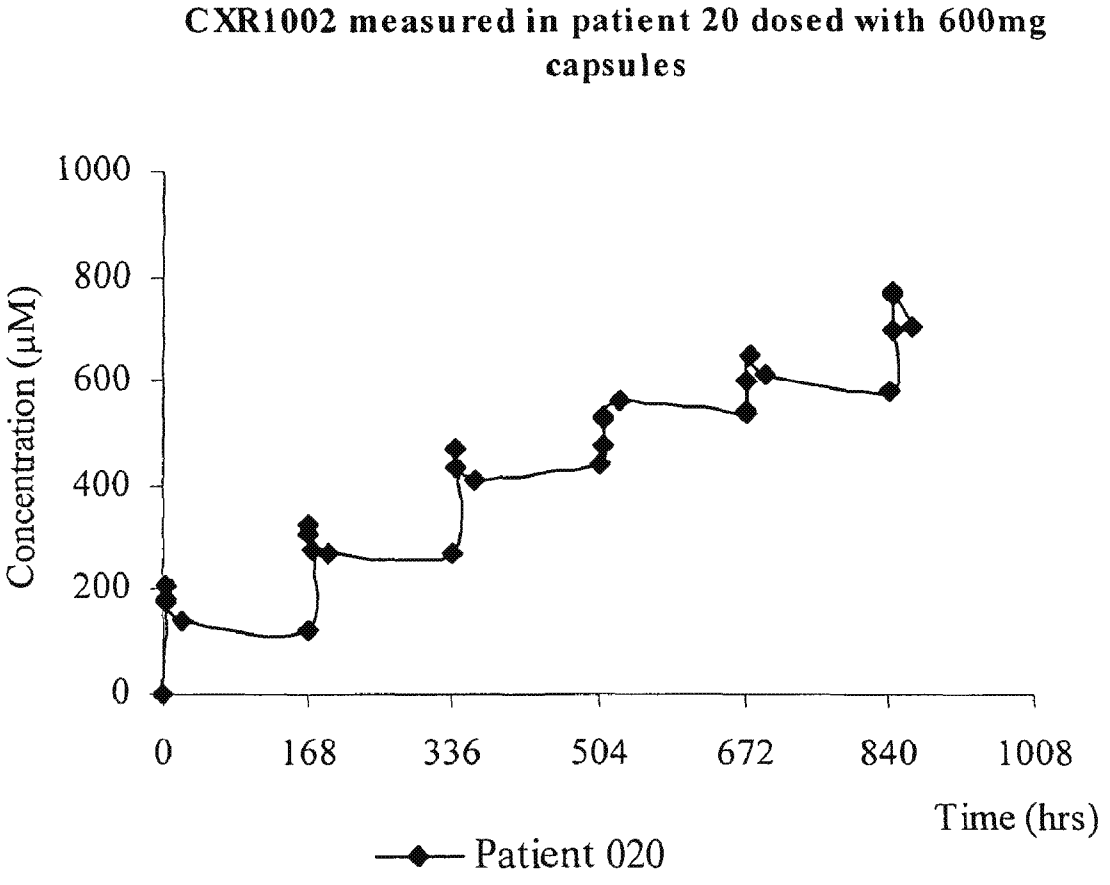

CXR1002 measured in patient 20 dosed with 600mg capsules
Concentration (μM)
1000
800
600
400
200
0
0
168
336
504
672
840
1008
Time (hrs)
Patient 020

Figure 69

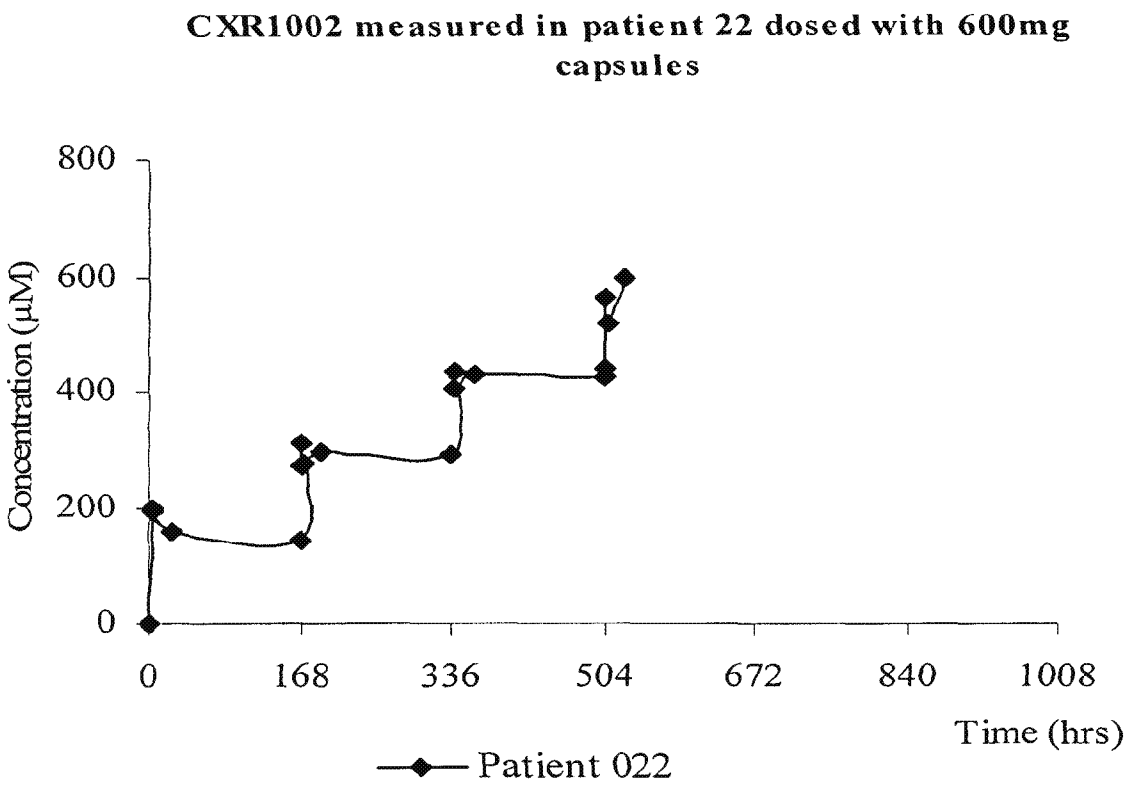

CXR1002 measured in patient 22 dosed with 600mg capsules
800
600
400
200
0
Concentration (μM)
0
168
336
504
672
840
1008
Time (hrs)
Patient 022

Figure 70

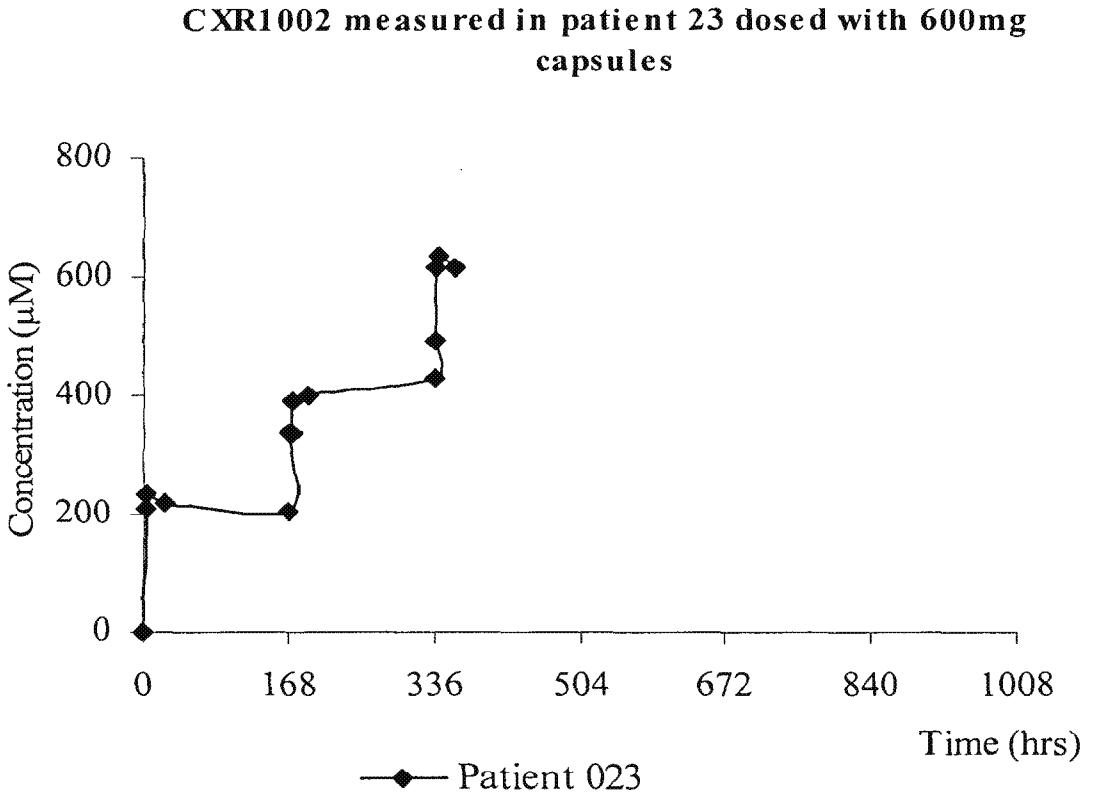

CXR1002 measured in patient 23 dosed with 600mg capsules
800
600
400
200
0
Concentration (μM)
0
168
336
504
672
840
1008
Time (hrs)
Patient 023

Figure 71

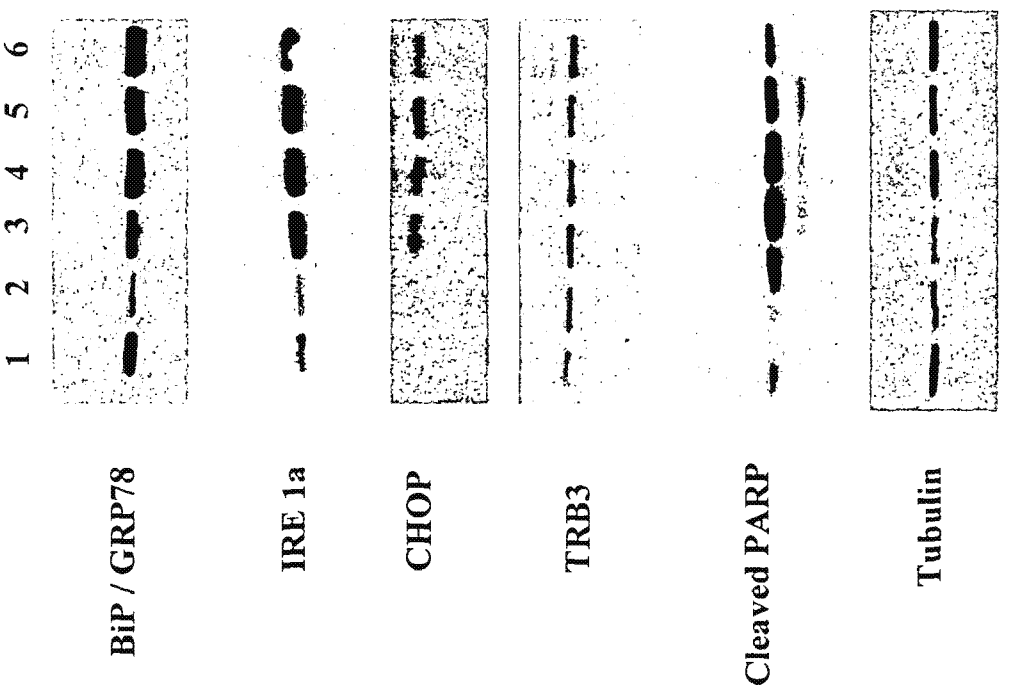
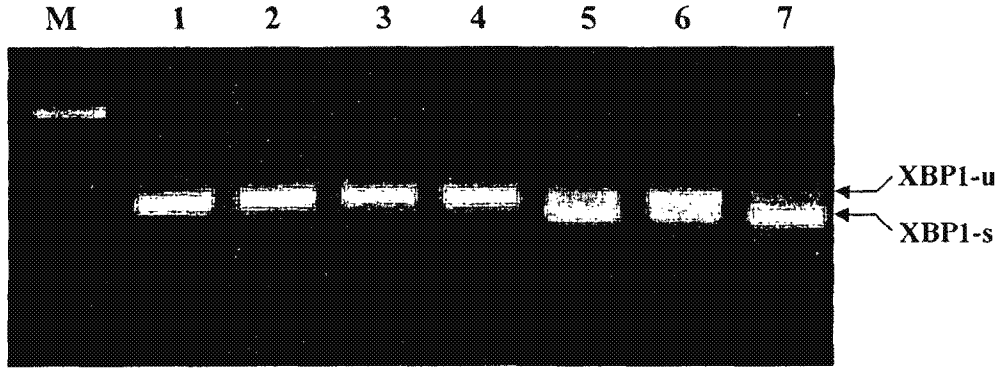


Figure 72

A: Panc-1 cells (IC50 > 1000 μM)



B: HepG2 cells (IC50: 380 μM)

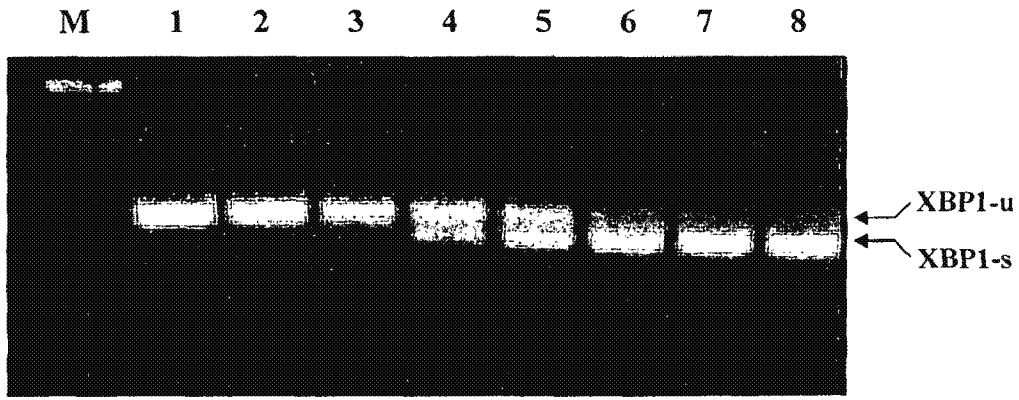


Figure 73

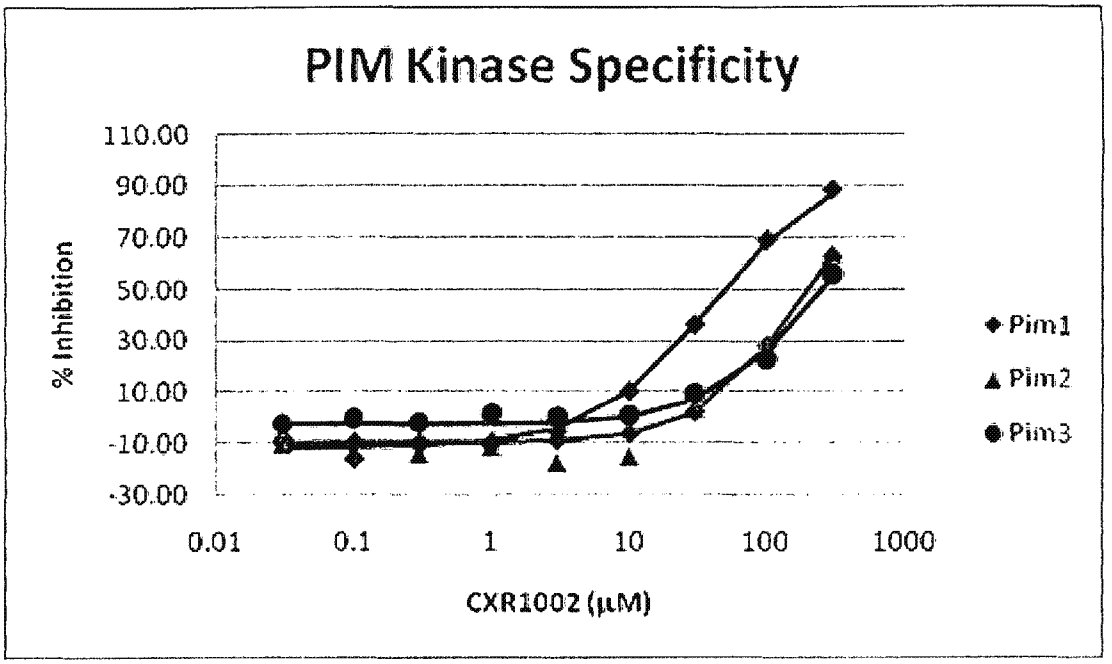

PIM Kinase Specificity
110.00
90.00
70.00
50.00
30.00
10.00
-10.00
-30.00
% Inhibition
0.01
0.1
1
10
100
1000
CXR1002 (μM)
Pim1
Pim2
Pim3

Figure 74

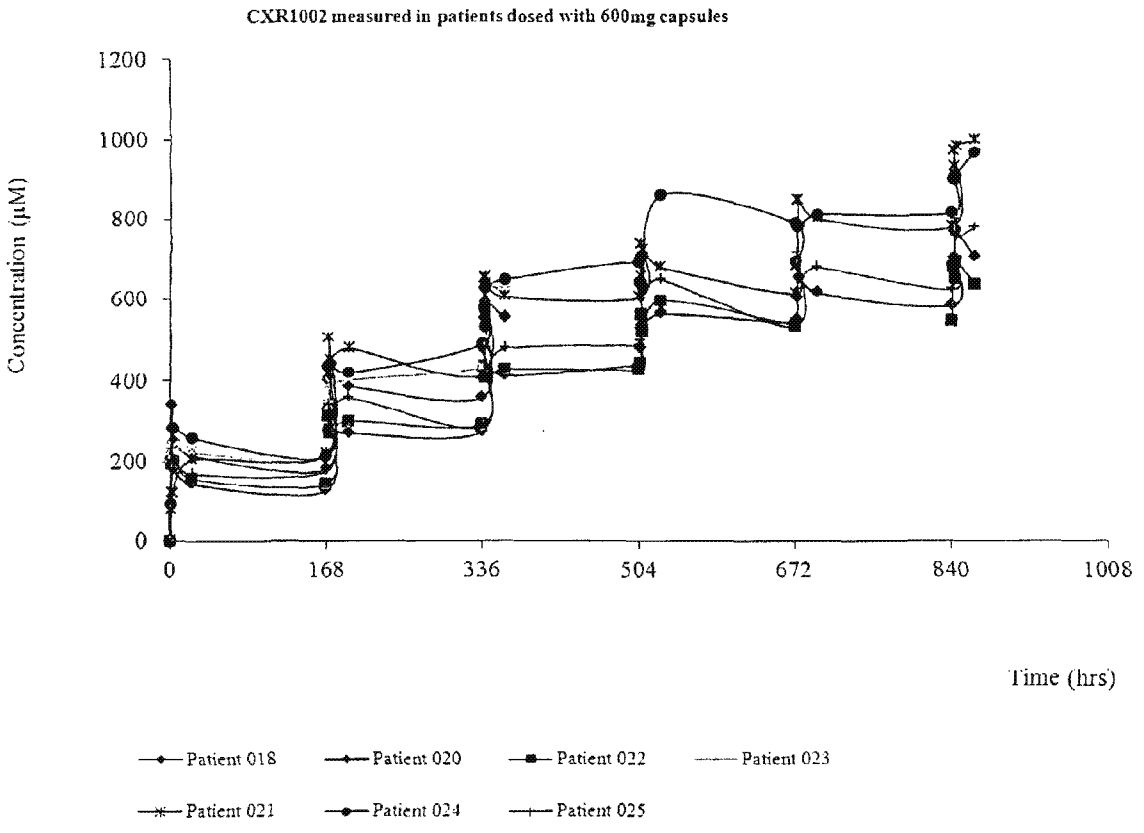

CXR1002 measured in patients dosed with 600mg capsules
Concentration (µM)
1200
1000
800
600
400
200
0
0
168
336
504
672
840
1008
Time (hrs)
Patient 018
Patient 020
Patient 022
Patient 023
Patient 021
Patient 024
Patient 025

Figure 75

Effects of Dose Increments on CXR1002 Plasma Exposure Level

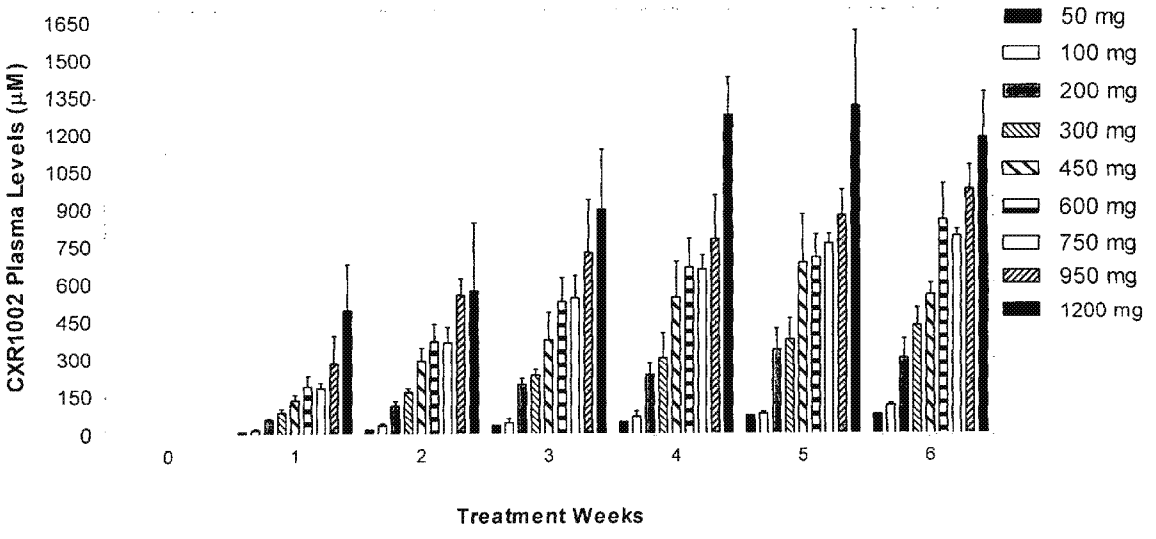


Figure 76

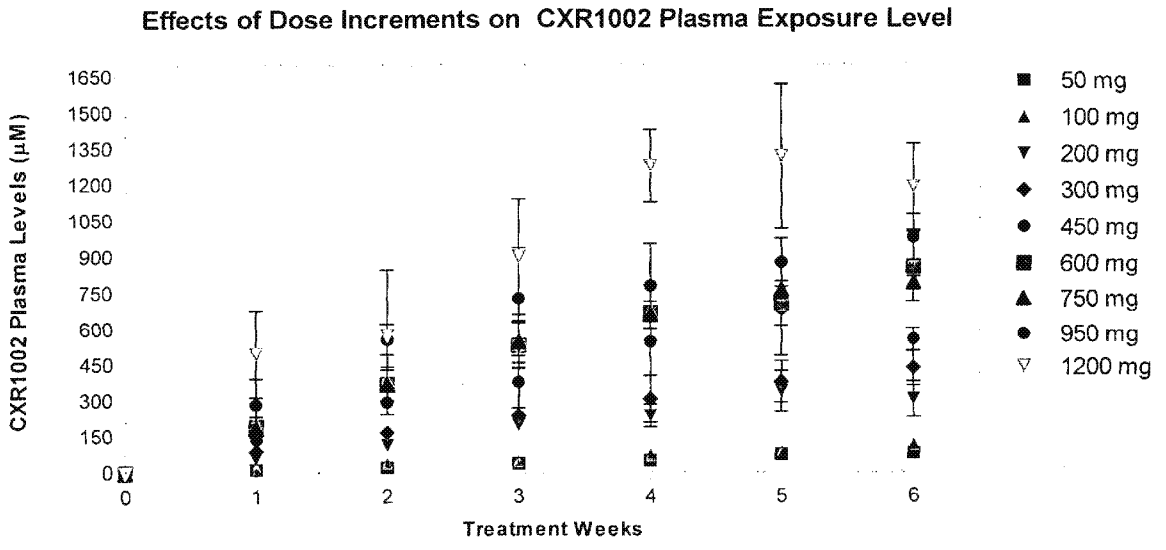

Effects of Dose Increments on CXR1002 Plasma Exposure Level
CXR1002 Plasma Levels (μM)
1650
1500
1350
1200
1050
900
750
600
450
300
150
0
0
1
2
3
4
5
6
Treatment Weeks
50 mg
100 mg
200 mg
300 mg
450 mg
600 mg
750 mg
950 mg
1200 mg

Figure 77

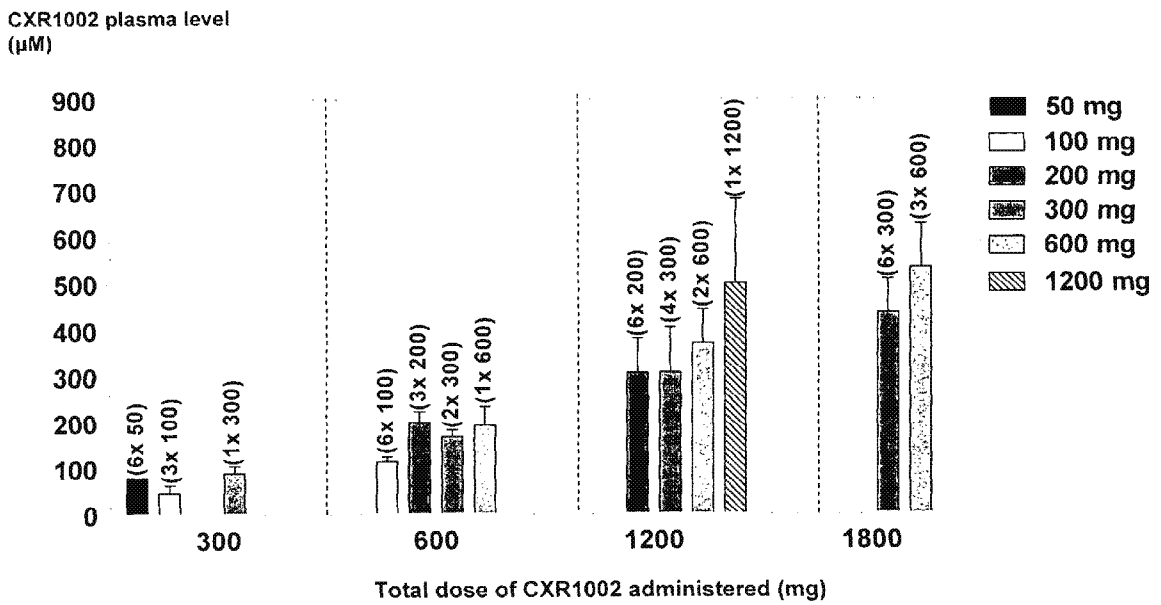

CXR1002 plasma level (μM)
900
800
700
600
500
400
300
200
100
0
(6x 50)
(3x 100)
(1x 300)
(6x 100)
(3x 200)
(2x 300)
(1x 600)
(6x 200)
(4x 300)
(2x 600)
(1x 1200)
(6x 300)
(3x 600)
300
600
1200
1800
Total dose of CXR1002 administered (mg)
50 mg
100 mg
200 mg
300 mg
600 mg
1200 mg

Figure 78

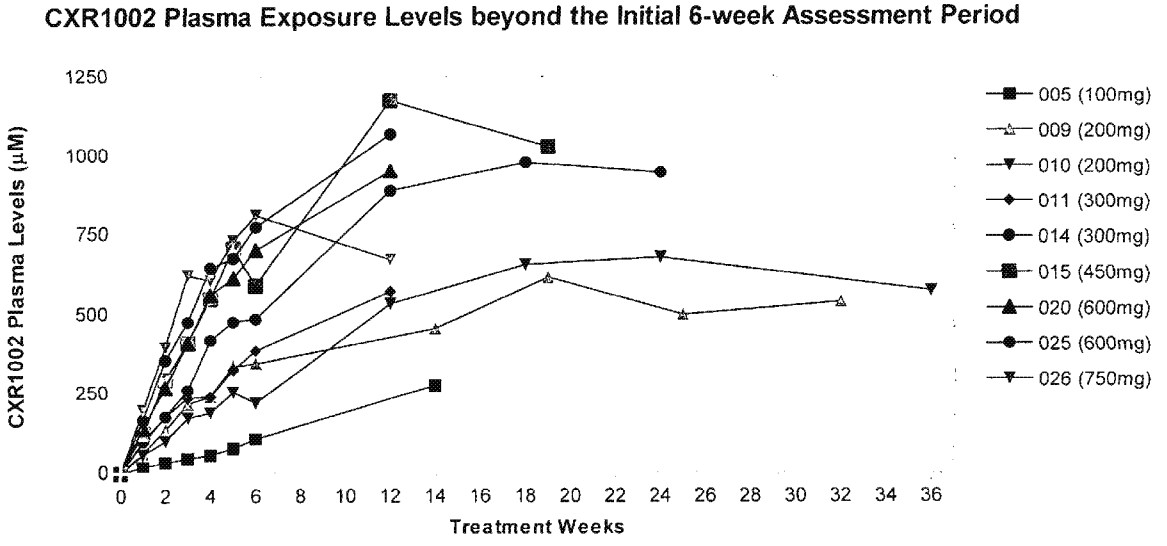

CXR1002 Plasma Exposure Levels beyond the Initial 6-week Assessment Period
CXR1002 Plasma Levels (µM)
1250
1000
750
500
250
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36
Treatment Weeks
005 (100mg)
009 (200mg)
010 (200mg)
011 (300mg)
014 (300mg)
015 (450mg)
020 (600mg)
025 (600mg)
026 (750mg)

Figure 79

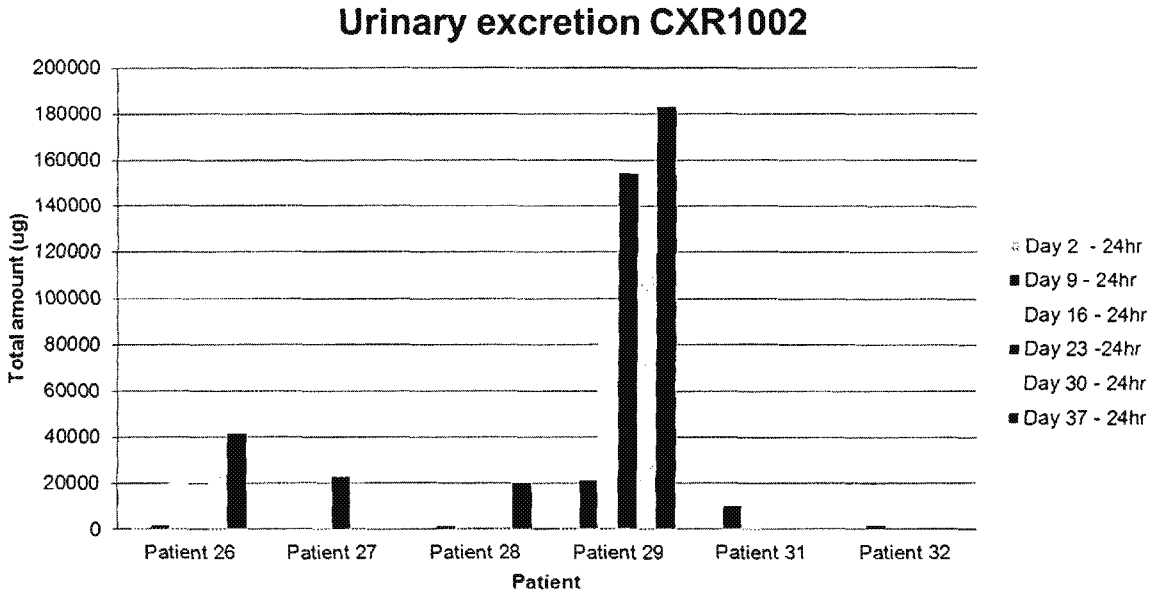

Urinary excretion CXR1002
Total amount (ug)
200000
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
Patient 26
Patient 27
Patient 28
Patient 29
Patient 31
Patient 32
Patient
Day 2 - 24hr
Day 9 - 24hr
Day 16 - 24hr
Day 23 -24hr
Day 30 - 24hr
Day 37 - 24hr

Figure 80

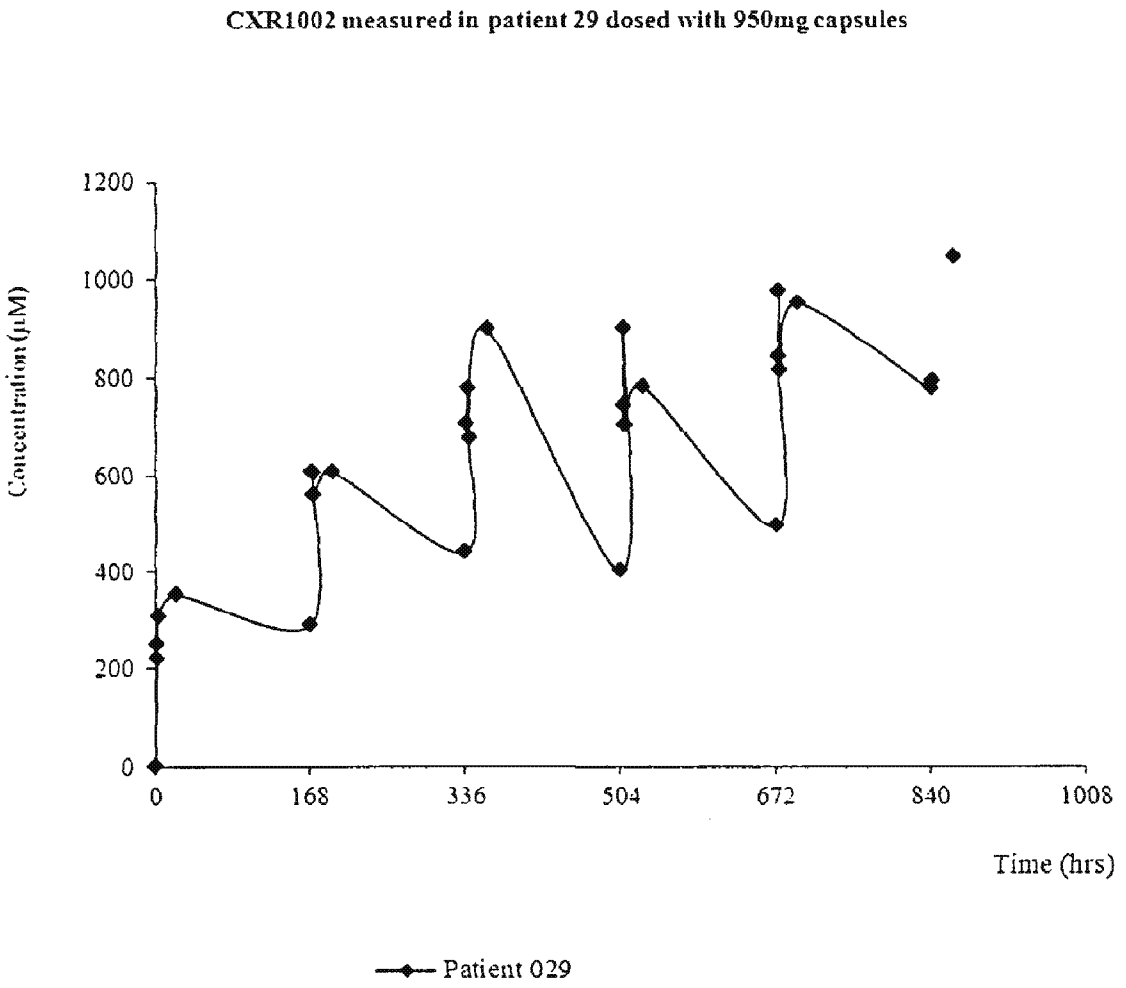

CXR1002 measured in patient 29 dosed with 950mg capsules
Concentration (µM)
1200
1000
800
600
400
200
0
0
168
336
504
672
840
1008
Time (hrs)
Patient 029

Figure 81

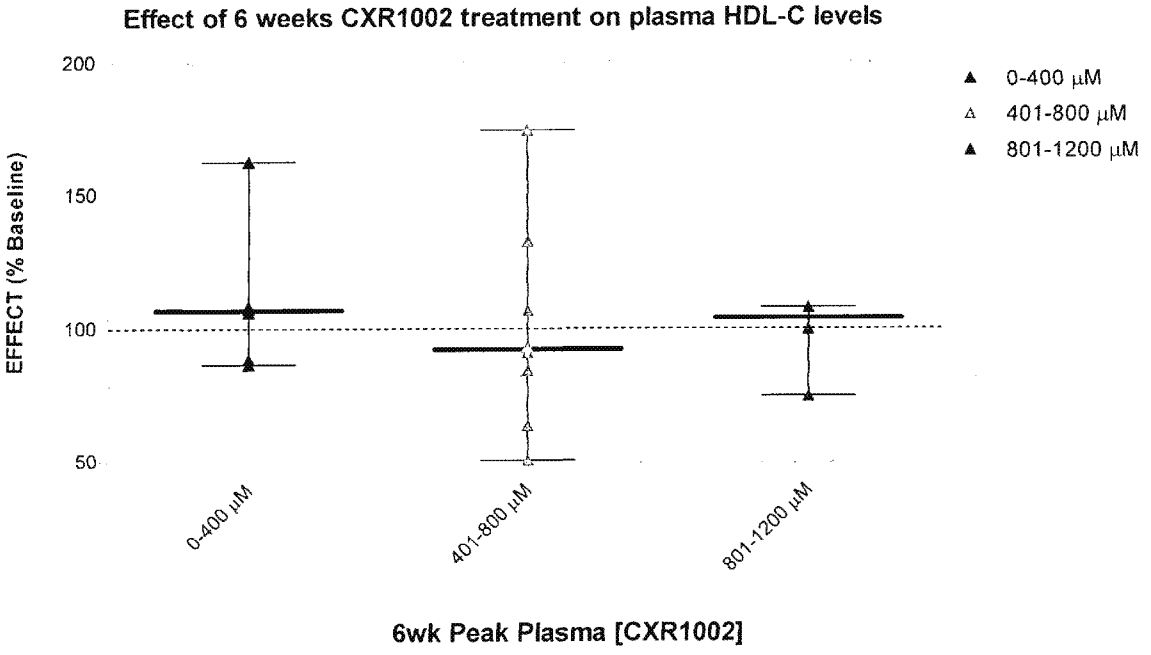
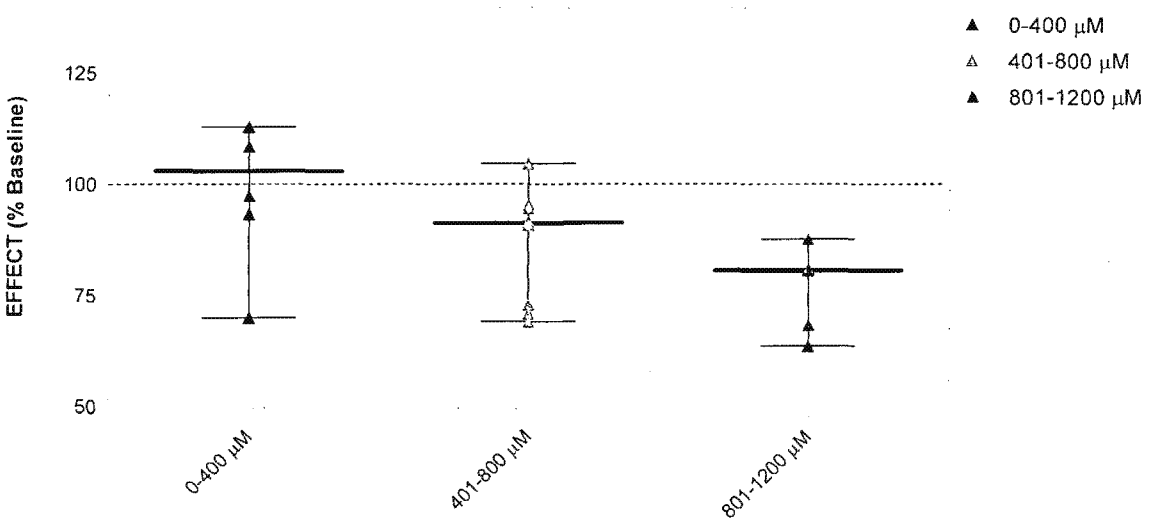

Effect of 6 weeks CXR1002 treatment on plasma HDL-C levels
EFFECT (% Baseline)
200
150
100
50
0-400 µM
401-800 µM
801-1200 µM
0-400 µM
401-800 µM
801-1200 µM
6wk Peak Plasma [CXR1002]

Figure 82

Effect of 6 weeks CXR1002 treatment on plasma LDL-C levels



6wk Peak Plasma [CXR1002]

Figure 83

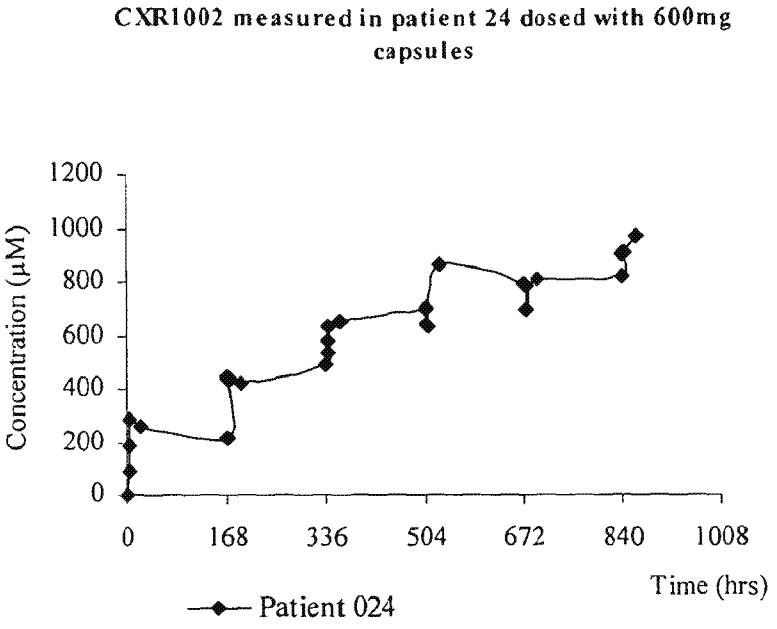


Figure 84

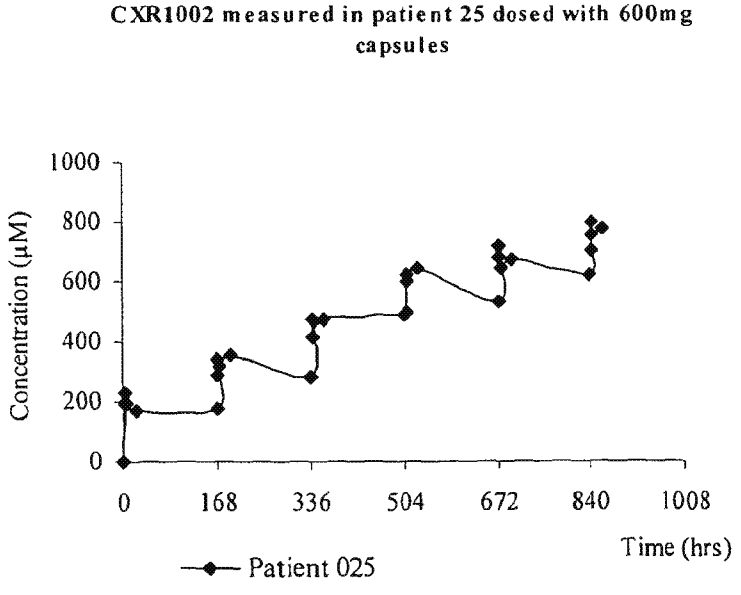


Figure 85

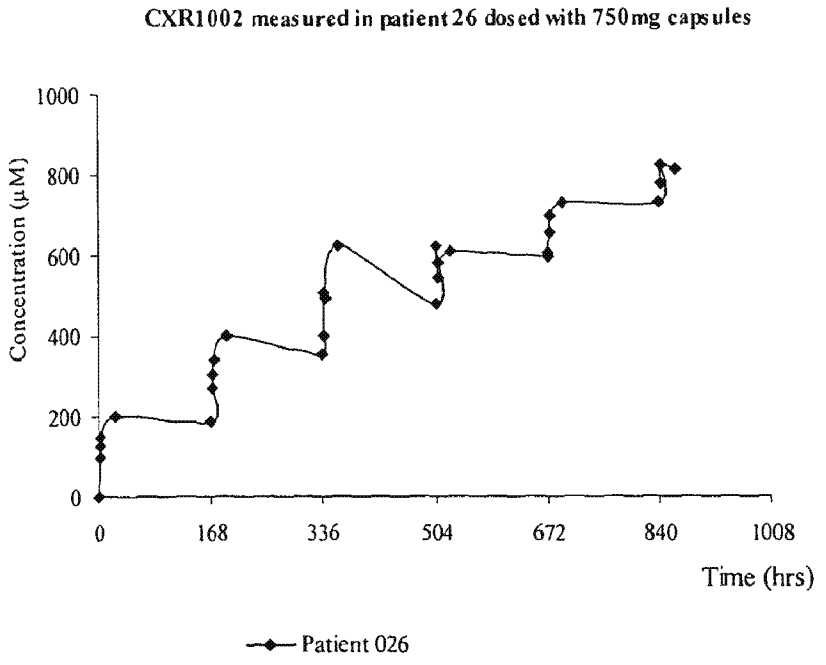


Figure 86

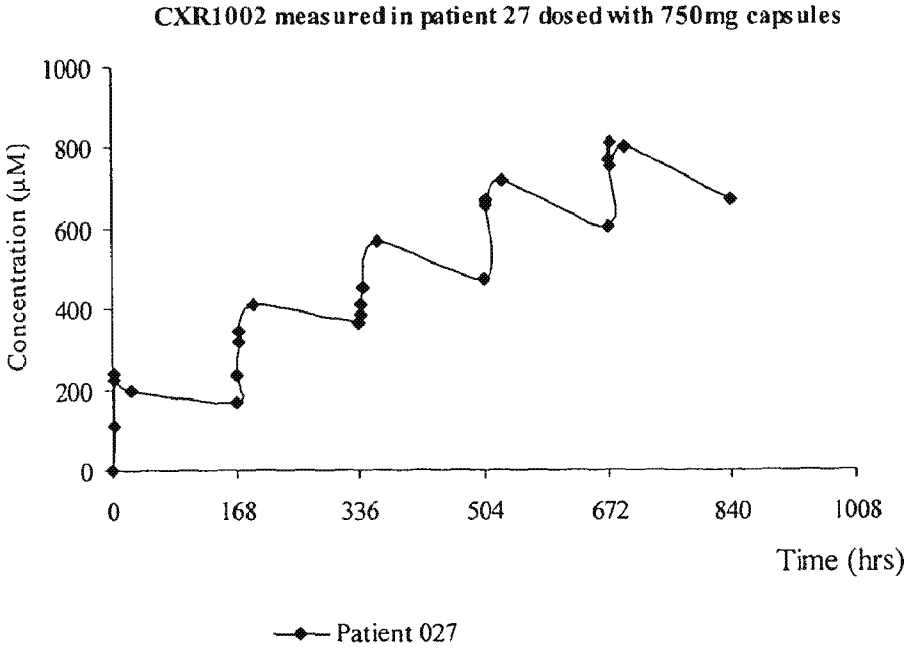


Figure 87

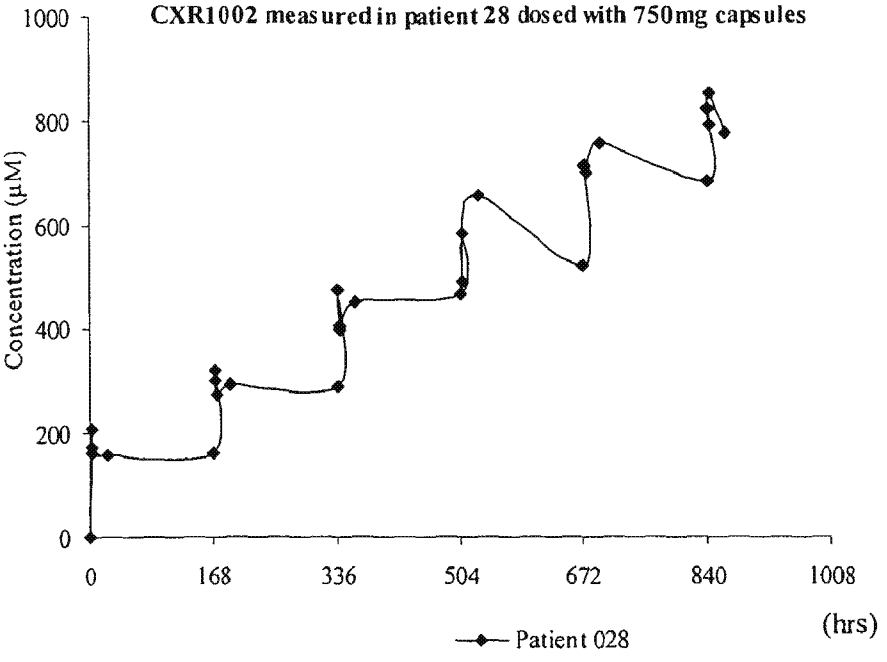


Figure 88

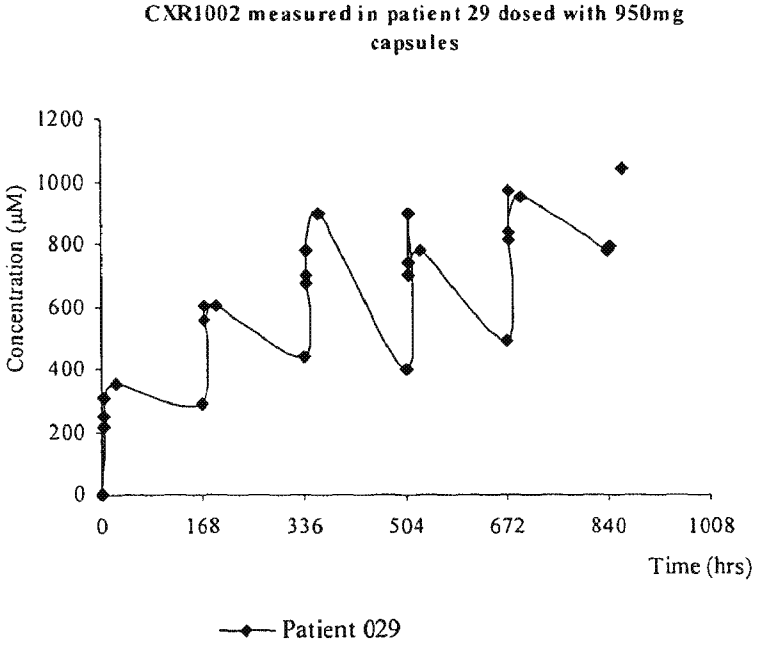


Figure 89

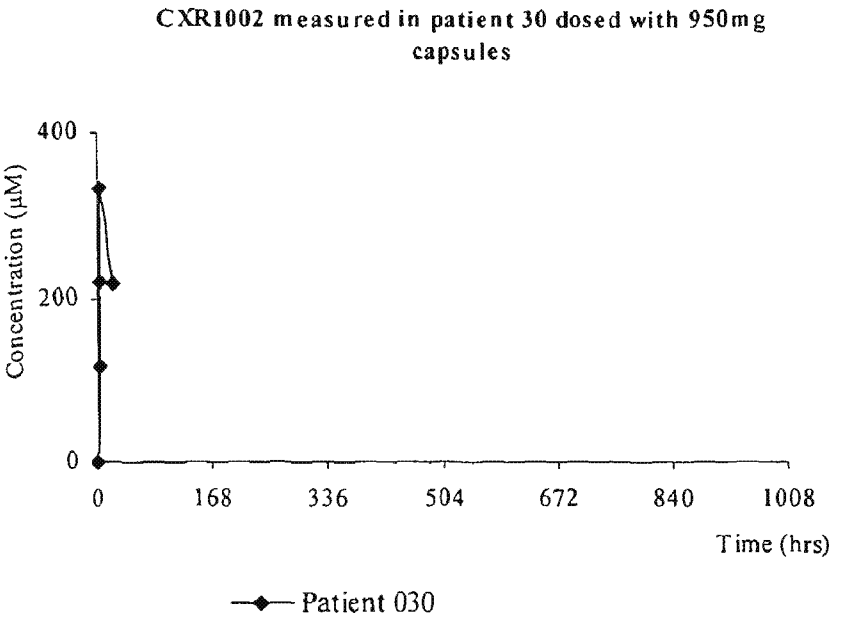


Figure 90

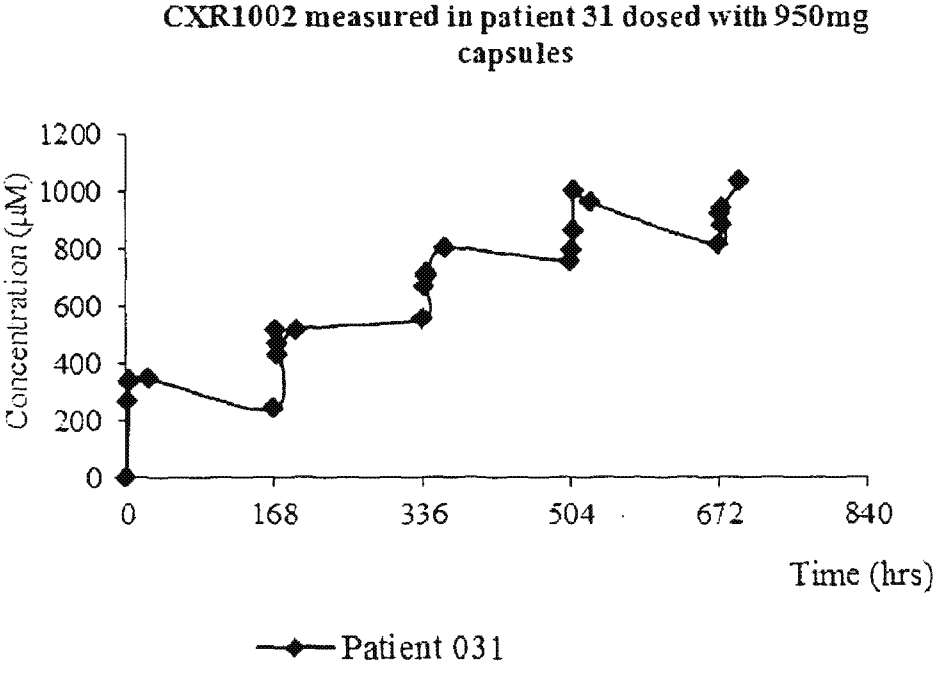


Figure 91

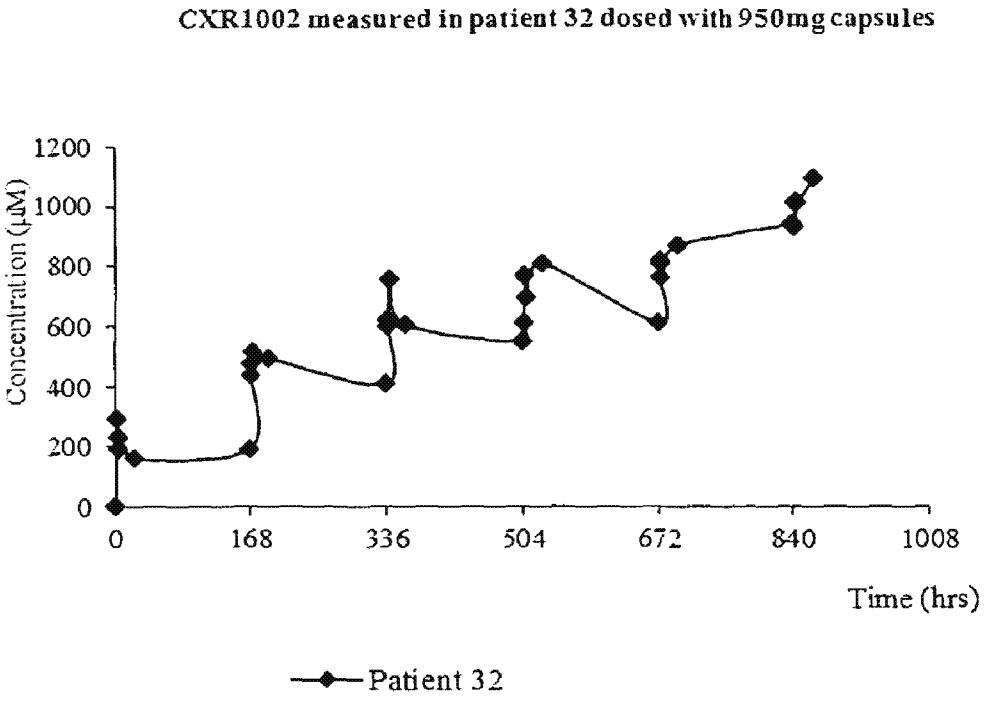


Figure 92

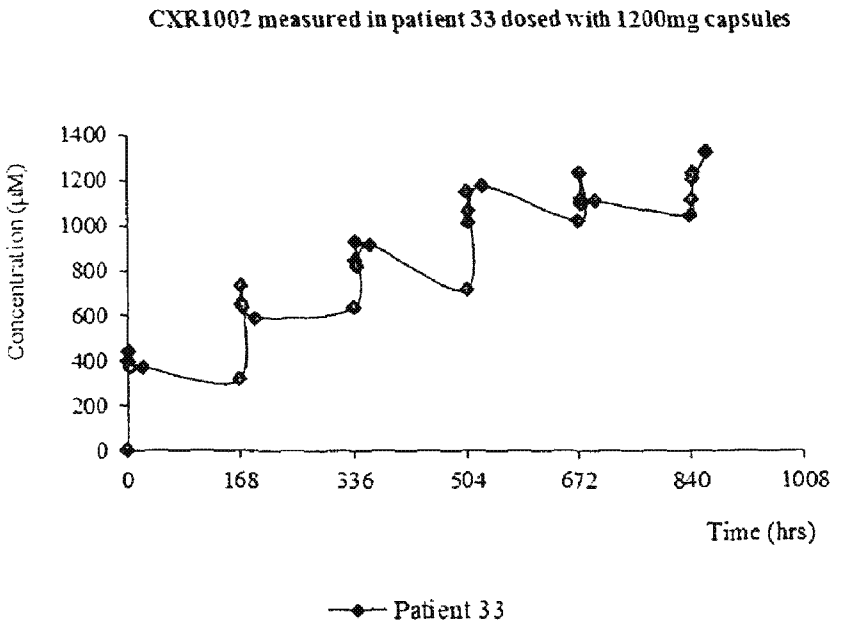


Figure 93

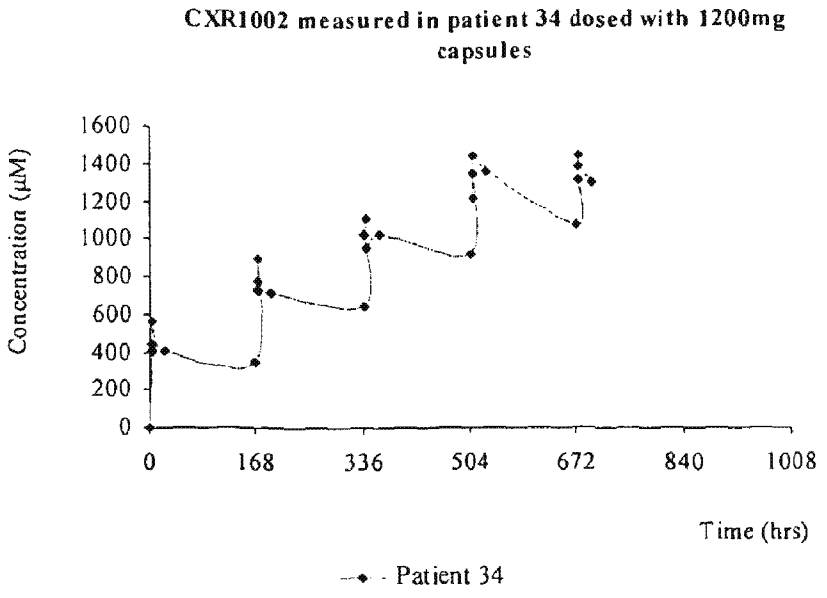


Figure 94

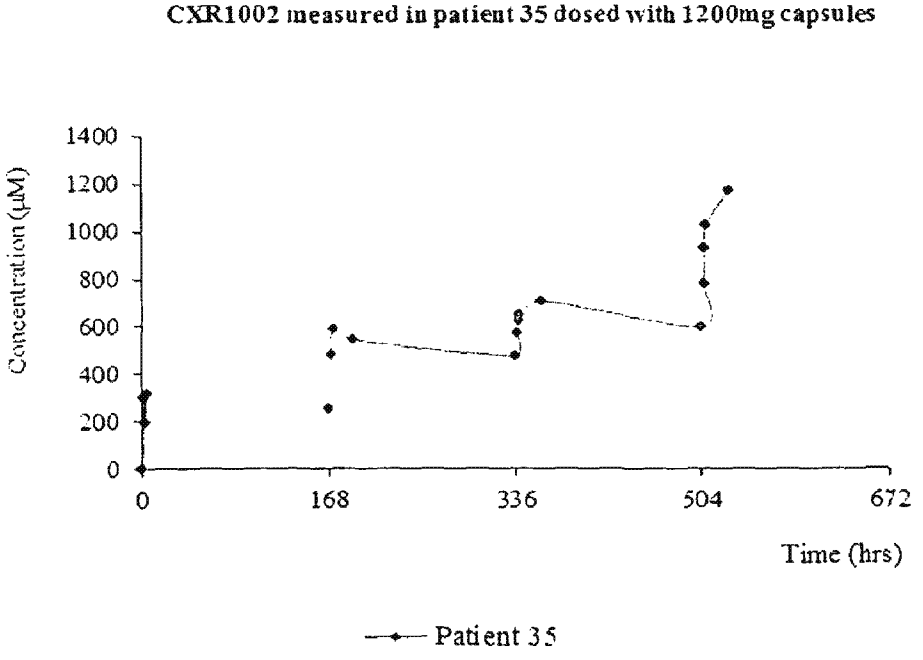


Figure 95

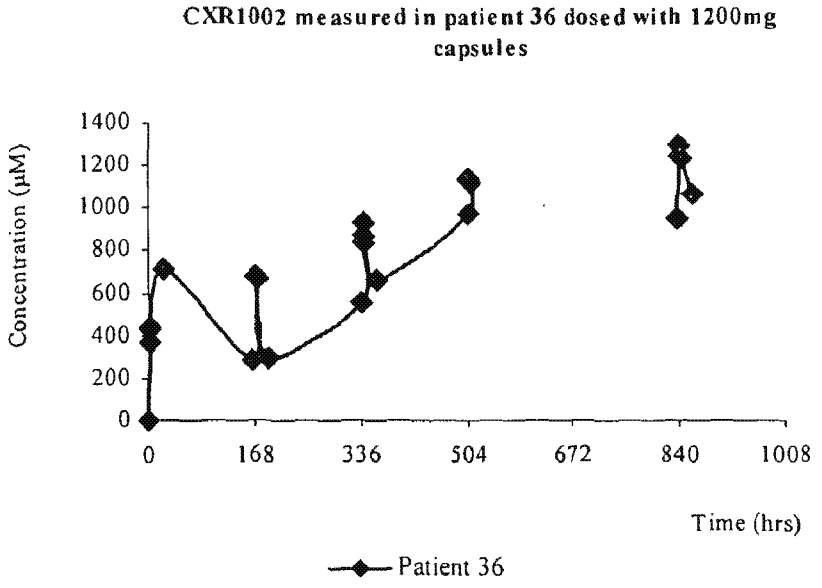


Figure 96

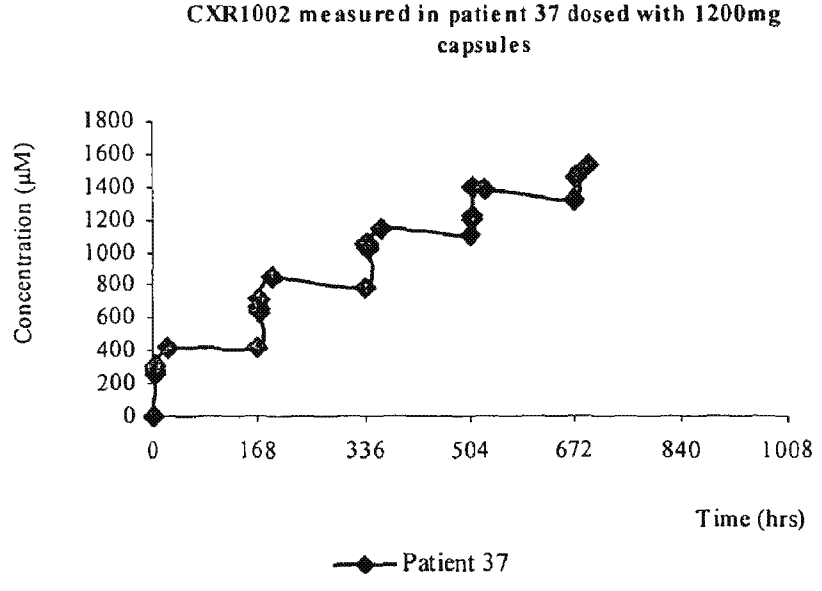


Figure 97

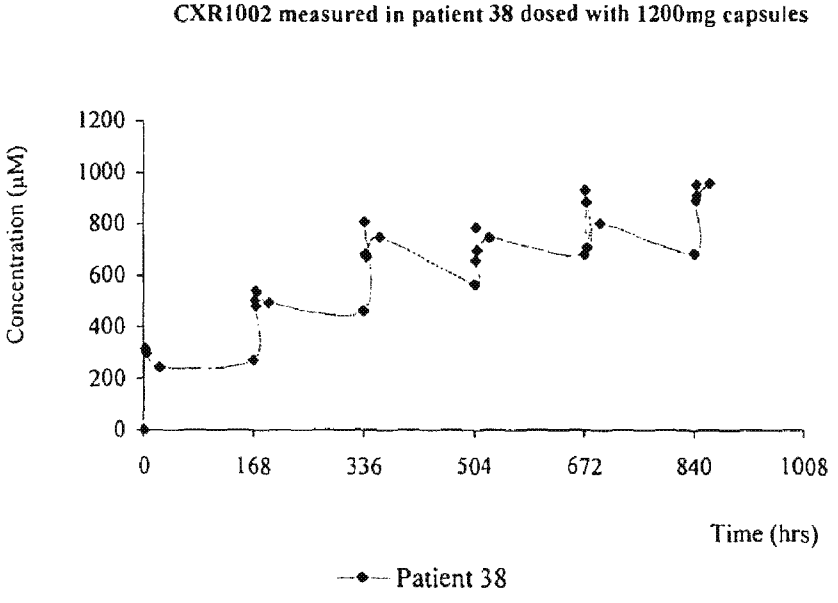


Figure 98

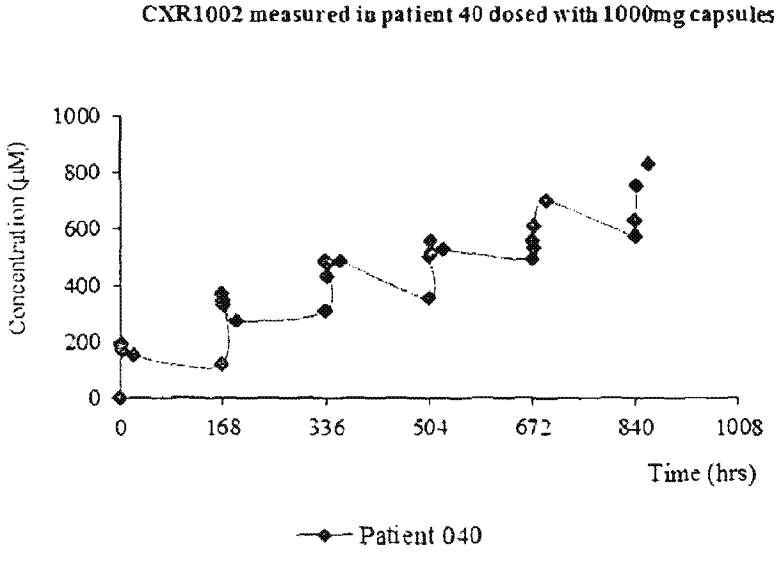


Figure 99

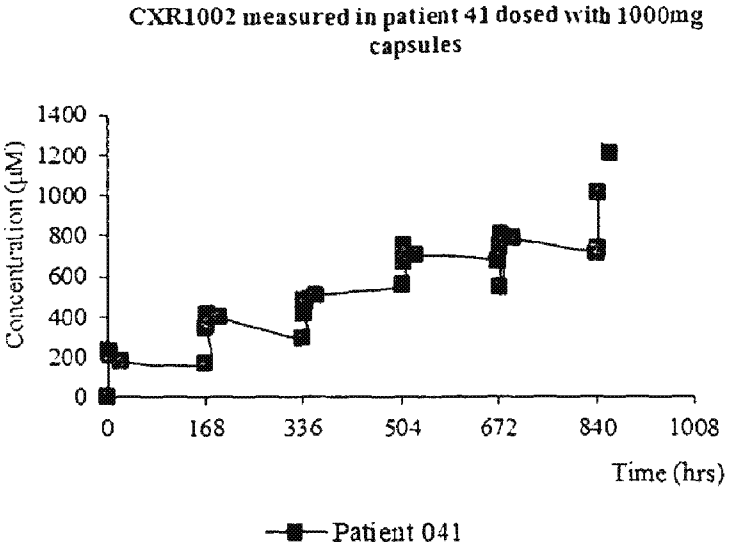


Figure 100

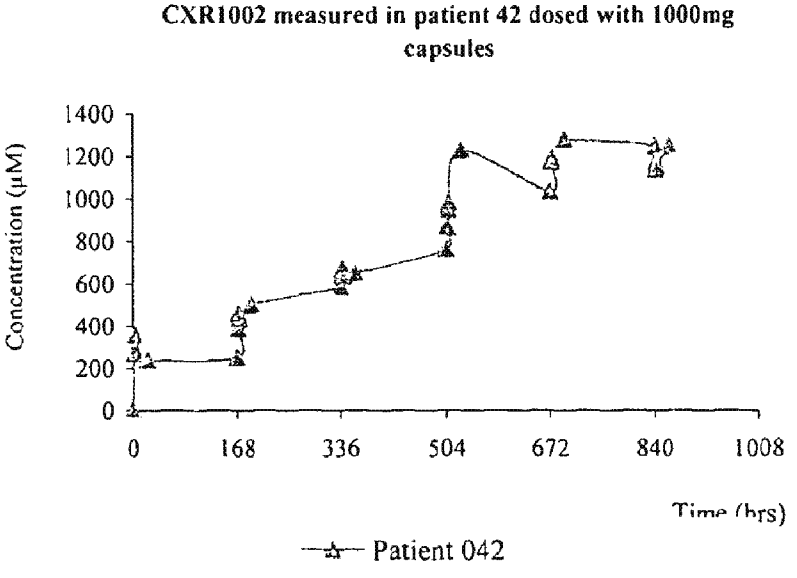
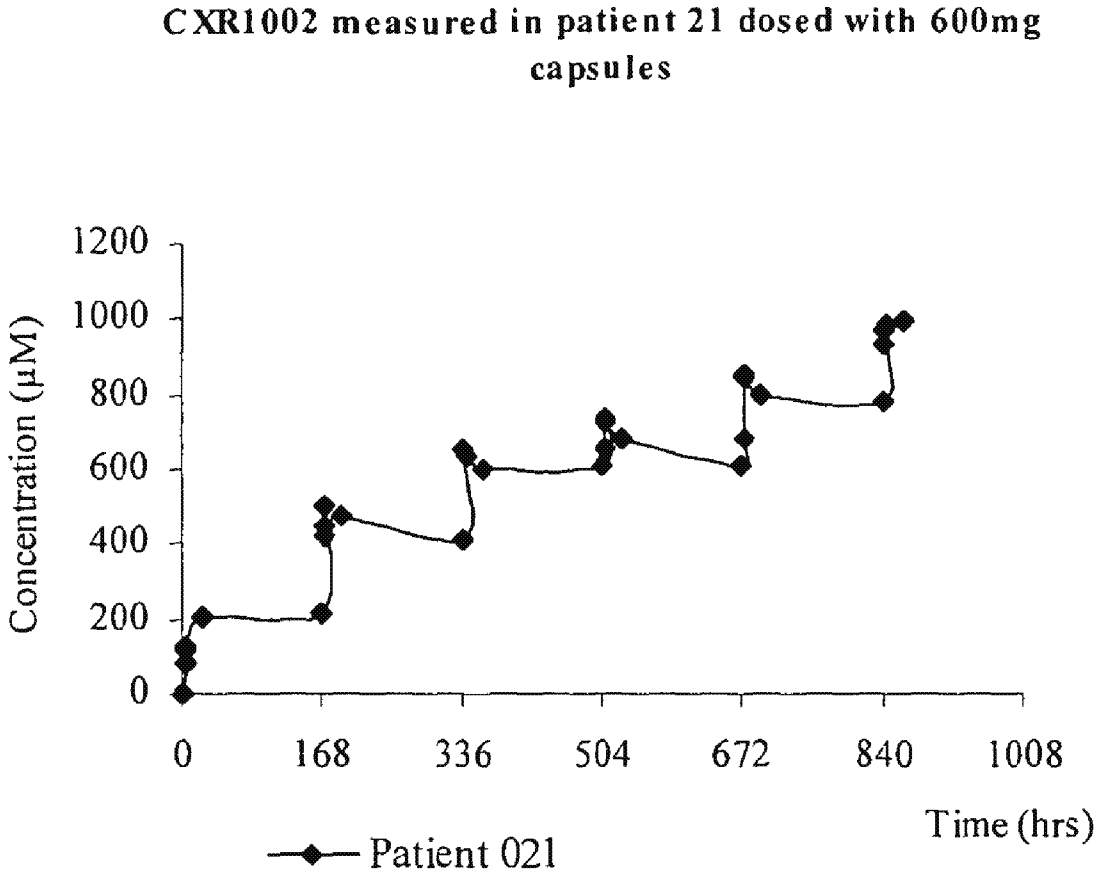


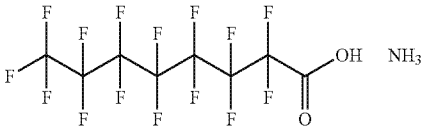
Figure 101


CXR1002 measured in patient 21 dosed with 600mg capsules
Concentration (µM)
1200
1000
800
600
400
200
0
0
168
336
504
672
840
1008
Time (hrs)
Patient 021

COMPOSITIONS COMPRISING PERFLUOROOCTANOIC ACID

[0001] The invention relates to compositions for treating cancer. In particular there is provided, doses, dosage regimes for the administration of Perfluorooctanoate (PFOA) and in particular, Ammonium Perfluorooctanoate (APFO) in the treatment of cancer.

[0002] Ammonium Perfluorooctanoate (APFO) has the molecular formula $C_8F_{15}O_2.H_4N$



[0003] APFO is the ammonium salt of straight chain perfluorooctanoic acid (PFOA). Commercially available ammonium perfluorooctanoate (APFO) is a mixture of approximately 75% straight chain and 25% various branched isomers.

[0004] Preliminary experiments to evaluate mode of action were performed using this mixture (APFO). We have previously described (WO 2004/019927 WO 2002/66028) the use of perfluorinated carboxylic acids for the treatment of cancer.

[0005] Subsequently, the purified straight chain isomer was obtained, and the results obtained with APFO were verified with this isomer (CXR1002).

[0006] CXR1002 is a fatty acid mimetic in that it interacts with fatty acid homeostasis and/or a fatty acid mediated pathway. Both CXR1002 and APFO isomers and also perfluoroalkyls of different chain lengths possess these properties. This has been demonstrated in Vanden Heuvel (1996) where it was shown that different nuclear hormone receptors were activated by PFOA and how this compared to natural fatty acid activation of the same receptors. Wolf (2008) showed a dose response of various chain length perfluoroalkyls against PPAR alpha (FIG. 3 of Wolf (2008)) in a transiently transfected COS-1 cell model to compare the C4 to C9 chain lengths.

[0007] It has now been shown that APFO and the CXR1002 isomer has additional mechanisms of action accounting for some of its anti-tumour effects.

[0008] APFO has been shown to cause Endoplasmic Reticulum (ER) stress (see Example 8). Endoplasmic reticulum stress induction has been shown to have an anti-tumour effect, including in pancreatic cancer, myeloma and thyroid cancer. For example, sorafenib, bortezomib and Hsp90 cause cell death by induction of ER stress pathways and bortezomib is used clinically to treat multiple myeloma and mantle cell lymphoma. Review articles discussing the association with ER stress and Cancer are Healy (2009), Strasser (2008) and Moenner (2007).

[0009] APFO has also been shown to have activity against PIM kinases (see Example 9). PIM kinases are cytoplasmic serine/threonine kinases that are known to be involved in regulation of apoptosis and cellular metabolism. Certain PIM kinases have been shown to be upregulated in cancers and as such their inhibition represents a mechanism of action by which CXR1002 can have an anti-tumour effect in conditions such as leukaemia, lymphoma, prostate cancer, colon cancer and pancreatic cancer. The below studies have shown this link:

[0010] Liver cancer: Gong (2009), Fujii (2005) and Wu (2010) have shown PIM-2 to promote tumorigenesis and PIM-3 to accelerate hepatocellular carcinoma development when induced by hepatocarcinogen.

[0011] Gastric cancer: Zhen (2008) and Warnecke-Eberz (2009) have shown overexpression of PIM-1 in gastric glands to be associated with lymph node metastases.

[0012] Head and neck cancer: Beier (2007) has shown PIM-1 overexpression in head and neck squamous cell carcinomas.

[0013] Colon cancer: Popivanova (2007) has shown PIM-3 to be aberrantly expressed in human colon cancer cells but not normal colon mucosa.

[0014] Pancreatic cancer: Li (2006), Chen (2009) and Reiser-Erkan (2008) have shown PIM-3 expression occurs in human pancreatic cancer but not normal cells and PIM-1 blockage using siRNA resensitises pancreatic cancer cells to apoptosis and PIM-1 levels correlate to clinicopathological parameters in pancreatic cancer.

[0015] Leukaemia/lymphoma: Adam (2006), Hammerman (2005), Cohen (2004), Hogan (2008), Lin (2010), Kim (2005), Chen (2008) and Brault (2010) have shown PIM-2 expression is increased in leukaemia/lymphoma, expression of PIM-1 and PIM-2 is dependent on Abl kinase activity and PIM-1 mediates homing and migration of malignant haematopoietic cells.

[0016] Oral cancer: Chiang (2006) and Choi (2010) have shown PIM-1 expression to be high in squamous cell carcinoma.

[0017] Prostrate cancer: Chen (2005), Mumenthaler (2009), He (2007), Xu (2005), Dai (2005) and Roh (2008) have shown PIM-1 overexpression in prostatic carcinoma.

[0018] Breast cancer: Roh (2008) has shown PIM-1 overexpression to convert mammary epithelia cells to become tumourgenic.

[0019] Adipocyte tumours: Nga (2010) has shown benign and malignant adipocytic tumours to have strong PIM-1 expression.

[0020] PIM kinases are constitutively active and their activity as shown above and in Amaravadi (2005) and Shah (2008) supports in vitro and in vivo human cell growth and survival.

[0021] APFO is a perfluorinated carboxylic acid that exerts its anti-tumour effects via multiple mechanisms of action. Previously it had been know that APFO acts by one or more peroxisome proliferator activated receptor (PPAR)-mediated mechanisms. PPARs are members of the nuclear hormone receptor family of transcription factors. They modulate DNA transcription by binding to specific peroxisome proliferator-response elements (PPREs) on target genes.

[0022] CXR1002 is a white, odourless solid that is freely soluble in water. CXR1002 and its family of compounds are extremely stable.

[0023] The investigational medicinal product being made in the clinical trials described in the examples consists of Size 1 white opaque gelatin capsules containing the active substance, CXR 1002. There is no bulking agent. One strength of capsule has been manufactured with a target strength of 50 mg of CXR1002 per capsule.

[0024] Laboratory studies have indicated that CXR1002 can interact with cells in a number of different ways which could be associated with its pharmacological effectiveness as

an anti-tumour agent. For example, CXR1002 is an agonist of PPARs and also induces ER-stress in tumour cells. CXR1002 has also been shown to have a range of biological effects probably related to its surfactant properties, including; alteration of cell membrane potential and cytostolic pH (Kleszczynski (2009)); induction of oxidative stress (Fernandez (2008)) that was closely linked to cell cycle arrest; dissipation of mitochondrial membrane potential (Hu (2009)) and dysregulation of gap-junctional intercellular communication (GJIC) and activation of extracellular receptor kinase (ERK) (Upham (2009)). CXR1002 is cytotoxic to tumour cells with an IC_{50} ranging upwards from 273 μM.

[0025] The data presented demonstrate that CXR1002 has anti-tumour activity both in vitro and in xenograft models. The mechanism of action, involving agonism of PPARs α and γ in association with neutral or inhibitory action on PPARδ, is distinct from those of currently available chemotherapeutic agents. In addition CXR1002 induces ER-stress in some cancer cell lines; this may be an effect that is related to its effects on PPARs. Furthermore CXR1002 is an inhibitor of the PIM kinase family of serine/threonine kinases. CXR1002 could provide anticancer activity against a range of tumour types. Humans have already received environmental exposure to CXR1002 and workers involved in the manufacture of APFO have been recorded as having serum concentrations as high as 275 μM without reported adverse effects. Furthermore, patients in the ongoing CXR1002-001 study have exposure in the 200 μM to 800 μM range after a few weeks of dosing with CXR1002. This level of exposure to cells in vitro or to a xenografted tumour would be expected to have a biological effect.

[0026] As of February 2011, 43 patients with advanced cancers from one Phase I study have received CXR1002. CXR1002 is not metabolised and dosing is accumulative. It is presumed that CXR1002 will eventually reach a steady state level after a number of doses, in an analogous way to its accumulative exposure in monkeys. The lack of metabolism of CXR1002 provides an advantage over other chemotherapeutic agents such that inter-patient variability in exposure is low as metabolism of the active ingredient at different rates in different patients is not an issue for CXR1002.

[0027] Significant occupational exposure to PFOA and its salts, including APFO, has occurred over many years and APFO has been found in the blood of workers exposed in the workplace. The dogma derived from studies such as these is that CXR1002 has a long serum half-life in humans (range=109 to 1308 days). Data from the CXR1002-001 clinical trial, demonstrate that after a single dose of CXR1002, the plasma level of the drug is constant over the 6 week sampling period, indicating that the half life is >6 weeks.

[0028] However, patients in the phase I study receiving >100 mg weekly dose have higher exposure after 6 weeks of dosing than the maximal values recorded in occupationally exposed workers.

[0029] A large database of experimental studies on the potential health hazards of APFO is available, as are recent toxicology reviews (USEPA (2005)), (Kennedy (2004)). In addition to toxicology studies in laboratory animals, the potential association of APFO exposure with health effects in fluorochemical production workers has been studied since 1976 through medical monitoring and epidemiological investigations (Ubel (1980)), (Olsen (1998)), (Olsen (2000)).

[0030] The majority of studies reported in the literature have used APFO itself, although some studies using other salts have also been described. The biological effects of APFO are thought to be due to its dissociation to form perfluorooctanoate (PFOA), the anionic form of perfluorooctanoic acid. Perfluorooctanoic acid and its salts are soluble in water and readily dissociates to the carboxylate anion, perfluorooctanoate (PFOA) (Kennedy (2004)).

[0031] The consensus is that, since the active constituent of each of these compounds is the perfluorooctanoate anion, these studies are directly comparable. An extensive toxicology and occupational health database already exists for this compound. Several studies of relevance have been commissioned by commercial companies but the reports are not in the public domain. However, the field has been thoroughly reviewed by Kennedy et al. (2004) and the USEPA (2005). In addition, key studies have been published in the scientific literature or are available through the USEPA public docket.

[0032] Most commercial studies on APFO/PFOA have used a commercial material e.g. FC-143 FLUORAD, which comprises 93-97% APFO and the remaining consisting of a mixture of Ammonium perfluoropentanoate, Ammonium perfluoroheptanoate and Ammonium perfluorohexanaote.

[0033] Unlike most other anti-tumour agents, PFOA is efficiently absorbed following oral exposure. It is not metabolised and is eliminated intact. PFOA exhibits only moderate acute oral toxicity. Signs and symptoms of toxicity include body weight loss, liver weight increase and liver effects as demonstrated by increased serum transaminase activity and diffuse hepatocellular hypertrophy accompanied, at higher doses, by acidophilic degeneration and/or necrosis of the liver. PFOA exhibits no teratogenic or foetotoxic effects in rats at doses below those causing maternal toxicity and there is no evidence of any adverse effects on reproductive success in a two-generation reproduction study. Two year cancer bioassays in rats resulted in increased incidence of benign tumours (adenomas) of the liver, pancreas (acinar cell) and testes (Leydig cell) at 300 ppm in the diet, but not at 30 ppm. A battery of tests for genotoxicity demonstrated that PFOA does not cause either point mutations or chromosomal aberrations.

[0034] None of the toxicology studies give any indication of changes in cardiovascular, central nervous system, respiratory or renal function induced by PFOA. Studies in rats have revealed no clinical signs that suggested adverse pharmacological effects. Furthermore, there was no evidence of such effects in a 26-week toxicity study in male cynomolgus monkeys.

[0035] Although no specific studies have been carried out in humans on the potential unwanted pharmacological effects of PFOA, there are no significant toxicities reported in workers with significant occupational exposure.

[0036] PFOA is well absorbed following oral exposure. After a single oral dose of ^{14}C-PFOA (11 mg/kg) to male rats at least 93% of total radioactivity was absorbed at 24 hrs (58). Following a single gavage administration to rats (25 mg/kg), peak blood levels were attained 1-2 hours after dosing (Kennedy (2004)). There was a clear sex difference in clearance. Blood levels in female rats showed >95% clearance 24 hrs after dosing, while blood levels in males remained relatively high throughout this period. The sex difference in clearance was even more marked 1 week after treatment, when blood levels in males remained relatively high and those in females had declined to very low levels.

[0037] Importantly, PFOA does not appear to accumulate in blood of female rats, since the blood profile of an oral dose of 25 mg/kg following 10 previous similar doses was quite similar to that observed after a single oral dose (Kennedy (2004)).

[0038] The amounts of PFOA deposited in the tissues of different species are inversely related to the species-specific rate of urinary excretion. In species which excrete PFOA slowly, the compound distributes primarily to the liver, plasma and the kidney and to a lesser extent other tissues of the body, including testis and ovary. For example, following 28 days gavage administration to male rats the major sites of deposition were the serum, liver and kidney. Little transfer to the brain occurs in adults. In female rats, the pattern of tissue deposition is dose-dependent. At 3 mg/kg more PFOA is deposited in the liver than the kidney whereas this is reversed at higher doses, suggesting the existence of a saturable renal excretory mechanism in the (female) rat (Kennedy (2004)).

[0039] There is no evidence that APFO is metabolised in mammals once dissociated to form perfluorooctanoate. However, analysis of five major drug metabolising cytochrome P450 (CYP) isozymes (CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4) indicated that CXR1002 is an inhibitor of CYP2C9, having an IC_{50} of 0.76 μM under the conditions used (unpublished data). Similar results were obtained using APFO, which had an IC_{50} of 0.78 μM towards CYP2C9.

[0040] The main factor determining the elimination rate of PFOA in different species is the rate of urinary excretion. In female rats, the extent of biliary excretion is <1.0% (Vanden (1991)).

[0041] The human renal clearance of PFOA has been evaluated in Japanese volunteers (Hasada (2005)). There were no significant differences in the renal clearance of PFOA with regard to sex, age group, medication, and medical or residential history.

[0042] To date, studies of PFOA have primarily related to the effects of the compound as a contaminant and occupational exposure in humans. Little is known regarding its safe and effective use as a therapeutic agent. Safe and efficacious dosages and therapeutic administration regimes have now been identified, specifically in relation to the treatment of cancer.

[0043] Furthermore, combinations of PFOA and other chemotherapeutic agents that are unexpectedly advantageous have also been identified as part of the invention.

[0044] APFO, and in particular the CXR1002 isomer has a large number of beneficial properties in comparison to existing chemotherapeutic agents. For example, CXR1002 is highly water soluble and as such is highly bioavailable. The high bioavailability is partially explained by CXR1002 possessing a long half life (shown to be greater than 6 weeks of half life in the clinical trials discussed in the examples). CXR1002 is now known not to be a substrate for human metabolism and as such dose and plasma concentration are closely linked and importantly variation between individuals is minimal (as there is no metabolism of CXR1002 there is no variability between individuals in metabolism). The slow clearance of CXR1002 means that a missed dose can be easily compensated for at a later date without an extensive loss of exposure to CXR1002. Due to the low variability of CXR1002 metabolism and clearance between individuals, dose strength and dose frequency required to achieve a desired plasma concentration is readily calculable by a skilled person because circulating plasma concentration can be reliably predicted from each dose taken.

[0045] CXR1002 has been shown in the clinical trials described in the examples to be orally bioavailable and this allows for simpler administration than current chemotherapeutic treatments (which are often given by intravenous administration), even to the point of allowing CXR1002 to be taken by patients outside of a hospital setting. In addition the CXR1002 capsule formulation has at least a 57 month shelf life that is commercially useful. The clinical trial work being conducted on CXR1002 has shown that CXR1002 is relatively non-toxic (at the doses examined to date CXR1002 does not cause toxicity commonly associated with anti-cancer drugs (no myelosuppresion, no anaemia, no transfusion requirement, no hair loss, mild or no effect on digestive system (individual variability apparent), no mouth ulcers, no skin problems, no lung effects, no heart effects, no neuropathy or nerve changes).

[0046] Although there is some reported nausea and vomiting with CXR1002, study subjects are not receiving concomitant anti-emetics, and these adverse events are of short duration.

[0047] Although CXR1002 causes liver enzyme changes in many toxicological test species (such as rats), the frequency of this in study subjects is low, with the predominant side effects being relatively mild including lethargy and mild gastrointestinal disturbance, nausea/vomiting and diarrhoea). The low toxicity of CXR1002 is supported by evaluation of pharmacodynamic markers in the clinical trials as discussed in the examples, which has shown there to be no significant changes.

[0048] The low toxicity profile and lack of metabolism allow CXR1002 to be used in combination with other therapeutic regimes with significant side-effects including cytotoxic chemotherapeutics and radiotherapy. Unlike other chemotherapeutics, CXR1002 can be used at the same time or prior to surgery with no wash out period required as CXR1002 would not exhibit the same side-effects as other chemotherapeutics on wound healing and immune response (due to the low toxicity of CXR1002).

[0049] Hence CXR1002 has been shown to possess significant advantages over other chemotherapeutics, these advantages allowing the specific compositions, dosage regimes and combination therapies to be identified and optimized as herein described.

[0050] In a first aspect of the invention there is provided a composition comprising between 10 mg and 2000 mg of an active ingredient per dosage unit, wherein the active ingredient is perfluorooctanoic acid (PFOA) or a derivative, salt or variant thereof.

[0051] By dosage unit we mean the unit of medicament administered to a patient at one time. For example, the dosage unit, or single dose may be administered by a single capsule/tablet, single injection, or single intravenous infusion, a single subcutaneous injection, or by a single procedure using other routes of administration, as discussed below. Alternatively, the single dose may be administered to the patient by two or more capsules/tablets or injections given simultaneously or sequentially to deliver the entire dose to the patient in the continuous, single and defined treatment period; by two or more intravenous infusions given simultaneously or sequentially to deliver the entire dose to the patient in the

continuous, single and defined treatment; or by multiple procedures using other routes of administration as discussed below.

[0052] Alternatively, the single dose to be administered to the patient can be delivered by a combination of routes to deliver the entire dose to the patient in the continuous, single and defined treatment.

[0053] The dosage unit may then be repeated at intervals of time such as a few hours, days, weeks, or months later.

[0054] Dosage units can be administered to patients in such a way that the patient receives a loading dose followed by one or more maintenance doses. For example the loading dose may be a high dose in order to quickly reach a desired plasma concentration and then subsequent maintenance doses are a lower dose than the loading dose in order to maintain the required plasma concentration.

[0055] By active ingredient we mean the molecule having the desired effect. In this case of this invention we primarily mean PFOA and derivatives, salts or variants thereof.

[0056] By variants and derivatives we mean any molecules of substantially identical chemical structure but including minor modifications that do not alter activity but may offer improved or alternative properties for formulation, such as formation into a salt.

[0057] In human therapy, the PFOA containing composition, and medicaments of the invention can be administered alone but will generally be administered in admixture with a suitable pharmaceutical excipient, diluent or carrier selected with regard to the intended route of administration and standard pharmaceutical practice.

[0058] For example, the PFOA containing composition, and medicaments of the invention can be administered orally, buccally or sublingually in the form of tablets, capsules, ovules, elixirs, solutions or suspensions, which may contain flavouring or colouring agents, for immediate-, delayed- or controlled-release applications. The PFOA containing composition, and medicaments of the invention may also be administered via intracavernosal injection.

[0059] Such tablets may contain excipients such as microcrystalline cellulose, lactose, sodium citrate, calcium carbonate, dibasic calcium phosphate and glycine, disintegrants such as starch (preferably corn, potato or tapioca starch), sodium starch glycollate, croscarmellose sodium and certain complex silicates, and granulation binders such as polyvinylpyrrolidone, hydroxypropylmethylcellulose (HPMC), hydroxy-propylcellulose (HPC), sucrose, gelatin and acacia. Additionally, lubricating agents such as magnesium stearate, stearic acid, glyceryl behenate and talc may be included.

[0060] Solid compositions of a similar type may also be employed as fillers in gelatin capsules. Preferred excipients in this regard include lactose, starch, cellulose, milk sugar or high molecular weight polyethylene glycols. For aqueous suspensions and/or elixirs, the PFOA containing composition, medicaments and pharmaceutical compositions of the invention may be combined with various sweetening or flavouring agents, colouring matter or dyes, with emulsifying and/or suspending agents and with diluents such as water, ethanol, propylene glycol and glycerin, and combinations thereof.

[0061] The PFOA containing composition, and medicaments of the invention can also be administered parenterally, for example, intravenously, intra-arterially, intraperitoneally, intra-thecally, intraventricularly, intrasternally, intracranially, intra-muscularly or subcutaneously, or they may be administered by infusion techniques. They are best used in the form of a sterile aqueous solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood. The aqueous solutions should be suitably buffered (preferably to a pH of from 3 to 9), if necessary. The preparation of suitable parenteral formulations under sterile conditions is readily accomplished by standard pharmaceutical techniques well-known to those skilled in the art.

[0062] Medicaments and pharmaceutical compositions suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The medicaments and pharmaceutical compositions may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

[0063] The PFOA containing composition, and medicaments of the invention can also be administered intranasally or by inhalation and are conveniently delivered in the form of a dry powder inhaler or an aerosol spray presentation from a pressurised container, pump, spray or nebuliser with the use of a suitable propellant, e.g. dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoro-ethane, a hydrofluoroalkane such as 1,1,1,2-tetrafluoroethane (HFA 134A3 or 1,1,1,2,3,3,3-heptafluoropropane (HFA 227EA3), carbon dioxide or other suitable gas. In the case of a pressurised aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. The pressurised container, pump, spray or nebuliser may contain a solution or suspension of the active agent, e.g. using a mixture of ethanol and the propellant as the solvent, which may additionally contain a lubricant, e.g. sorbitan trioleate. Capsules and cartridges (made, for example, from gelatin) for use in an inhaler or insufflator may be formulated to contain a powder mix of a PFOA containing composition, of the invention and a suitable powder base such as lactose or starch.

[0064] Aerosol or dry powder formulations are preferably arranged so that each metered dose or "puff" contains an effective amount of an agent or polynucleotide of the invention for delivery to the patient. It will be appreciated that the overall daily dose with an aerosol will vary from patient to patient, and may be administered in a single dose or, more usually, in divided doses throughout the day.

[0065] Alternatively, the PFOA containing composition, and medicaments of the invention can be administered in the form of a suppository or pessary, or they may be applied topically in the form of a lotion, solution, cream, gel, ointment or dusting powder. The PFOA containing composition, and medicaments of the invention may also be transdermally administered, for example, by the use of a skin patch. They may also be administered by the ocular route, particularly for treating diseases of the eye.

[0066] For ophthalmic use, the PFOA containing composition, and medicaments of the invention can be formulated as micronised suspensions in isotonic, pH adjusted, sterile saline, or, preferably, as solutions in isotonic, pH adjusted,

sterile saline, optionally in combination with a preservative such as a benzylalkonium chloride. Alternatively, they may be formulated in an ointment such as petrolatum.

[0067] For application topically to the skin, the PFOA containing composition, and medicaments of the invention can be formulated as a suitable ointment containing the active agent suspended or dissolved in, for example, a mixture with one or more of the following: mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene polyoxypropylene agent, emulsifying wax and water. Alternatively, they can be formulated as a suitable lotion or cream, suspended or dissolved in, for example, a mixture of one or more of the following: mineral oil, sorbitan monostearate, a polyethylene glycol, liquid paraffin, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

[0068] Formulations suitable for topical administration in the mouth include lozenges comprising the active ingredient in a flavoured basis, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouth-washes comprising the active ingredient in a suitable liquid carrier.

[0069] Generally, in humans, oral or parenteral administration of the PFOA containing composition, medicaments and pharmaceutical compositions of the invention is the preferred route, being the most convenient.

[0070] For veterinary use, the PFOA containing composition, and medicaments of the invention are administered as a suitably acceptable formulation in accordance with normal veterinary practice and the veterinary surgeon will determine the dosing regimen and route of administration which will be most appropriate for a particular animal.

[0071] The PFOA containing composition, as defined herein may be formulated as described in the accompanying Examples.

[0072] Preferably the PFOA is ammonium perfluorooctanoic acid (APFO), the ammonium salt.

[0073] The composition may comprise any effective amount of active ingredient, this may be between 10 mg and 2000 mg of active ingredient per dosage unit, and preferably is between 50 mg and 1000 mg. Advantageously it is 1000 mg. Conveniently, the dosage unit contains an amount of active ingredient per dosage unit selected from 10 mg, 20 mg, 25 mg, 50 mg, 100 mg, 200 mg, 300 mg, 400 mg, 450 mg, 600 mg, 750 mg, 950 mg, 1000 mg and 1200 mg.

[0074] Alternatively, the composition may comprise between 10-50 mg, 10-75 mg, 10-100 mg, 10-200 mg, 10-300 mg, 10-400 mg, 10-600 mg, 10-750 mg, 10-950 mg, 10-1000 mg, 10-1200 mg, 50-75 mg, 50-100 mg, 50-200 mg, 50-300 mg, 50-450 mg, 50-600 mg, 50-750 mg, 50-950 mg, 50-1000 mg, 50-1200 mg, 75-100 mg, 75-200 mg, 75-300 mg, 75-450 mg, 75-600 mg, 75-750 mg, 75-950 mg, 75-1000 mg, 75-1200 mg, 100-200 mg, 100-300 mg, 100-450 mg, 100-600 mg, 100-750 mg, 100-950 mg, 100-1000 mg, 100-1200 mg, 200-300 mg, 200-450 mg, 200-600 mg, 200-750 mg, 200-950 mg, 200-1000 mg, 200-1200 mg, 300-450 mg, 300-600 mg, 300-750 mg, 300-950 mg, 300-1000 mg, 300-1200 mg, 400-600 mg, 400-750 mg, 400-950 mg, 400-1000 mg, 400-1200 mg, 450-600 mg, 450-750 mg, 450-950 mg, 450-1000 mg, 450-1200 mg, 600-750 mg, 600-950 mg, 600-1000 mg, 600-1200 mg, 700-950 mg, 700-1000 mg, 700-1200 mg, 950-1000 mg, 950-1200 mg and 1000-1200 mg

[0075] Preferably there is 400-600 mg of active ingredient. More preferably there is 400-1200 mg of active ingredient. Most preferably there is 1000 mg of active ingredient.

[0076] Conveniently, the composition is pharmaceutically acceptable, and may optionally contain a pharmaceutically acceptable excipient, diluent, carrier or filler.

[0077] In a second aspect of the invention there is provided a composition as defined in the first aspect of the invention for use as a medicine.

[0078] In a third aspect of the invention there is provided a composition as defined in the first aspect of the invention for use in the treatment of cancer.

[0079] By "treatment" we include the meanings that tumour size is reduced and/or further tumour growth is retarded and/or prevented and/or the tumour is killed. We also include the reduction of other symptoms associated with the cancer being treated such as (but not limited to) a reduction in pain, cachexia and metastasis. The treatment may incorporate multiple aspects including chemotherapy, surgery and radiotherapy. The composition of the invention may be used on its own as a chemotherapeutic or with any other treatment for cancer, including before, during and after any other treatment type.

[0080] By 'treatment' we include both therapeutic and prophylactic treatment of a subject/patient. The term 'prophylactic' is used to encompass the use of composition described herein which either prevents or reduces the likelihood of the occurrence or development of cancer in a patient or subject.

[0081] A 'therapeutically effective amount', or 'effective amount', or 'therapeutically effective', as used herein, refers to that amount which provides a therapeutic effect for a given condition and administration regimen. This is a predetermined quantity of active material calculated to produce a desired therapeutic effect in association with the required additive and diluent, i.e. a carrier or administration vehicle. Further, it is intended to mean an amount sufficient to reduce or prevent a clinically significant deficit in the activity, function and response of the host. Alternatively, a therapeutically effective amount is sufficient to cause an improvement in a clinically significant condition in a host.

[0082] In a fourth aspect of the invention there is provided a use of a composition as defined in the first aspect of the invention in the manufacture of a medicament for the treatment of cancer.

[0083] In a fifth aspect of the invention there is provided a method of treating cancer comprising administering an effective amount of a composition as defined in the first aspect of the invention. Preferably the effective amount is between 10 and 2000 mg per dose, preferably between 50 and 600 mg per dose, and more preferably between 50 and 1200 mg per dose. Alternatively the effective amount is between 1 and 20 mg/kg, preferably between 1 and 7 mg/kg.

[0084] As is appreciated by those skilled in the art, the precise amount of a compound may vary depending on its specific activity. Suitable dosage amounts may contain a predetermined quantity of active composition calculated to produce the desired therapeutic effect in association with the required diluent. In the methods and use for manufacture of compositions of the invention, a therapeutically effective amount of the active component is provided. A therapeutically effective amount can be determined by the ordinary skilled medical or veterinary worker based on patient characteristics, such as age, weight, sex, condition, complications, other diseases, etc., as is well known in the art.

[0085] In a particularly preferred embodiment, the amount of the active ingredient administered to a patient is approximately between: 0.02 mg/kg to 0.10 mg/kg; or 0.10 mg to 0.20 mg/kg; or 0.20 mg to 0.30 mg/kg; or 0.30 mg to 0.40 mg/kg; or 0.40 mg to 0.50 mg/kg; or 0.50 mg to 0.60 mg/kg; or 0.60 mg to 0.70 mg/kg; or 0.70 mg to 0.80 mg/kg; or 0.80 mg to 0.90 mg/kg; or 0.90 mg to 1.00 mg/kg; or 1.00 mg to 1.10 mg/kg; or 1.10 mg to 1.20 mg/kg; or 1.20 mg to 1.30 mg/kg; or 1.30 mg to 1.40 mg/kg; or 1.40 mg to 1.50 mg/kg; or 1.50 mg to 1.60 mg/kg; or 1.60 mg to 1.70 mg/kg; or 1.70 mg to 1.80 mg/kg; or 1.80 mg to 1.90 mg/kg; or 1.90 mg to 2.00 mg/kg; or 2.00 mg/kg to 2.10 mg/kg; or 2.10 mg to 2.20 mg/kg; or 2.20 mg to 2.30 mg/kg; or 2.30 mg to 2.40 mg/kg; or 2.40 mg to 2.50 mg/kg; or 2.50 mg to 2.60 mg/kg; or 2.60 mg to 2.70 mg/kg; or 2.70 mg to 2.80 mg/kg; or 2.80 mg to 2.90 mg/kg; or 2.90 mg to 3.00 mg/kg; or 3.00 mg to 3.10 mg/kg; or 3.10 mg to 3.20 mg/kg; or 3.20 mg to 3.30 mg/kg; or 3.30 mg to 3.40 mg/kg; or 3.40 mg to 3.50 mg/kg; or 3.50 mg to 3.60 mg/kg; or 3.60 mg to 3.70 mg/kg; or 3.70 mg to 3.80 mg/kg; or 3.80 mg to 3.90 mg/kg; or 3.90 mg to 4.00 mg/kg; or 4.00 mg to 4.10 mg/kg; or 4.10 mg to 4.20 mg/kg; or 4.20 mg to 4.30 mg/kg; or 4.30 mg to 4.40 mg/kg; or 4.40 mg to 4.50 mg/kg; or 4.50 mg to 4.60 mg/kg; or 4.60 mg to 4.70 mg/kg; or 4.70 mg to 4.80 mg/kg; or 4.80 mg to 4.90 mg/kg; or 4.90 mg to 5.00 mg/kg; or 5.00 mg/kg to 6.00 mg/kg; or 6.00 mg to 7.00 mg/kg; or 7.00 mg to 8.00 mg/kg; or 8.00 mg to 9.00 mg/kg; or 9.00 mg to 10.00 mg/kg; or 10.00 mg to 11.00 mg/kg; or 11.00 mg to 12.00 mg/kg; or 12.00 mg to 13.00 mg/kg; or 13.00 mg to 14.00 mg/kg; or 14.00 mg to 15.00 mg/kg; or 15.00 mg to 16.00 mg/kg; or 16.00 mg to 17.00 mg/kg; or 17.00 mg to 18.00 mg/kg; or 18.00 mg to 19.00 mg/kg; or 19.00 mg to 20.00 mg/kg.

[0086] A composition, use or method of any of the third to fifth aspects wherein the treatment comprises the step of administering to a patient in need thereof an effective amount of the composition, in a single dosage at a frequency of once or twice per week (weekly or semi-weekly). Conveniently, the single dosage is administered at a frequency of less than once per week, preferably fortnightly or once per six weeks or less.

[0087] The dosage may be administered as a higher loading dose followed by one or more lower maintenance doses.

[0088] In a sixth aspect of the invention there is provided a therapeutic system for the treatment of cancer comprising administration of a composition as defined in the first aspect in a single dosage of between 10 mg and 2000 mg at a frequency of once per week or less.

[0089] By therapeutic system we mean a system of administering compositions to a patient in an effective manner to treat a specific disease. The system may be characterised by the dosages to be administered, the intervals between dosages and the methods of administration, or combinations thereof. The system may also be interchangeably known as a dosage regime.

[0090] Preferably, the dosage is between 200 mg and 1200 mg. Conveniently, the dosage is selected from 10 mg, 50 mg, 100 mg, 200 mg, 300 mg, 450 mg, 600 mg, 750 mg, 950 mg, 1000 mg and 1200 mg.

[0091] Alternatively, the dosage is selected from 1 mg/kg to 7 mg/kg.

[0092] Preferably, the dosage frequency is once per six weeks or less.

[0093] In the third to sixth aspects of the invention, the cancer may be selected from pancreatic cancer, ovarian cancer, breast cancer, prostate cancer, liver cancer, chondrosarcoma, lung cancer, head and neck cancer, colon cancer, sarcoma, leukaemia, lymphoma, kidney cancer, thyroid cancer and brain cancers such as glioblastoma.

[0094] In a seventh aspect of the invention there is provided a composition comprising perfluorooctanoic acid (PFOA) or a salt, derivative or variant thereof; and a further chemotherapeutic agent. Alternatively, there is provided a composition comprising an active ingredient as defined in the first aspect and a further chemotherapeutic agent.

[0095] Preferably, the further chemotherapeutic is selected from Doxorubicin, Gemcitabine, Roscovitine, Rapamycin, 5-FU, PARP inhibitors, kinase inhibitors including PIM kinase inhibitors and MAP kinase inhibitors, Hsp90 inhibitors (including Geldanamycin), proteasome inhibitors (including Bortezomib) and HDAC inhibitors (including SAHA); and prodrugs thereof.

[0096] Preferably, the further chemotherapeutic is present in an individually effective dose.

[0097] By individually effective dose we mean the dose at which the further chemotherapeutic is known to be effective when administered on its own.

[0098] Alternatively, the further chemotherapeutic is present in a lower than individually effective dose.

[0099] By lower than individually effective dose we mean a dose which is lower than that which is known to be the effective dose when the further chemotherapeutic is administered on its own. In other words, a lower dose than normal is administered because the combination provides a synergistic effect. This has the effect of reducing the administration of chemotherapeutics with unpleasant or dangerous side effects.

[0100] In an eighth aspect of the invention there is provided a composition as defined in the seventh aspect for use as a medicine.

[0101] In a ninth aspect there is provided a composition as defined in the seventh aspect for use in the treatment of cancer.

[0102] In a tenth aspect there is provided a use of a composition as defined in the seventh aspect in the manufacture of a medicament for the treatment of cancer.

[0103] In an eleventh aspect there is provided a method of treating cancer comprising administering an effective amount of a composition as defined in the seventh aspect.

[0104] In a twelfth aspect there is provided a therapeutic system for the treatment of cancer comprising a combination of component (i) a composition as defined in the first aspect; and (ii) a further chemotherapeutic agent, the components (i) and (ii) being provided for the use in the treatment of cancer and wherein components (i) and (ii) are administered in combination with one another.

[0105] By "in combination with one another" regarding the PFOA and chemotherapeutic agent treatments we include the meaning not only that the PFOA and chemotherapeutic agents are administered simultaneously, but also that they are administered separately and sequentially.

[0106] In one embodiment, administration of component (i) precedes administration of component (ii). In an alternative embodiment, administration of component (ii) precedes administration of component (i). In a further alternative embodiment, administration of component (i) occurs at the same time as administration of component (ii).

[0107] It is envisaged that the components may be administered in any order depending on individual circumstances including, need, drug availability, administration routes used.

Preferably the PFOA and chemotherapeutic agents are administered between 0 and 24 hours apart with either the PFOA or the chemotherapeutic being administered first.

[0108] Preferably, the further chemotherapeutic of the therapeutic system is selected from Doxorubicin, Gemcitabine, Roscovitine, Rapamycin, 5-FU, PARP inhibitors, kinase inhibitors including PIM kinase inhibitors and MAP kinase inhibitors, Hsp90 inhibitors (including Geldanamycin), proteasome inhibitors (including Bortezomib) and HDAC inhibitors (including SAHA); and prodrugs thereof.

[0109] In particular chemotherapeutics that enhance or complement the mechanisms of action of the composition of the invention (CXR1002) are preferred e.g. Hsp90 inhibitors, proteasome inhibitors and HDAC inhibitors.

[0110] Hsp90 inhibitors, including geldanamycin, target the chaperone Hsp90 and promote ubiquitin-dependent proteasomal degradation of proteins, leading to ER stress. Bortezomib, a proteasome inhibitor, also promotes the accumulation of aggregated, ubiquitinated proteins in the ER and therefore also cause ER stress. HDAC inhibitors have been shown to act synergistically with bortezomib, indicating that they may be id useful together with agents that induce ER stress (such as CXR1002). PIM kinase inhibition can restore sensitivity to FLT3 and BCR/ABL mutations that confer resistance to tyrosine kinase inhibitors.

[0111] In the ninth to twelfth aspects, the cancer may be selected from pancreatic cancer, ovarian cancer, breast cancer, prostate cancer, liver cancer, chondrosarcoma, lung cancer, head and neck cancer, colon cancer, sarcoma, leukaemia, lymphoma, kidney cancer, thyroid cancer and brain cancers such as glioblastoma.

[0112] In one embodiment when the cancer is pancreatic cancer, the further chemotherapeutic is selected from Doxorubicin, Gemcitabine, Geldanamycin and Roscovitine.

[0113] In an alternative embodiment, when the cancer is chondrosarcoma, the further chemotherapeutic is Gemcitabine.

[0114] In a further embodiment, when the cancer is ovarian cancer, the further chemotherapeutic is selected from Doxorubicin, Gemcitabine, Geldanamycin, Roscovitine, Rapamycin and 5-FU or pro-drugs thereof.

[0115] In a yet further embodiment, when the cancer is prostate cancer, the further chemotherapeutic is selected from Doxorubicin, Geldanamycin and Roscovitine

[0116] In another embodiment, when the cancer is breast cancer, the further chemotherapeutic is 5-FU or pro-drugs thereof.

[0117] In an alternative embodiment, when the cancer is liver cancer, the further chemotherapeutic is selected from Gemcitabine, Geldanamycin, Roscovitine and Rapamycin.

[0118] In a thirteenth aspect of the invention there is provided a kit of parts comprising:

(i) a composition as defined in the first embodiment; and

(ii) a further chemotherapeutic agent.

[0119] The kit may optionally comprise:

(iii) means of administering (i) and (ii) to a patient, wherein the administration may be at the same time or in succession.

[0120] Preferably the further chemotherapeutic agent of the kit is selected from Doxorubicin, Gemcitabine, Roscovitine, Rapamycin, 5-FU, PARP inhibitors, kinase inhibitors including PIM kinase inhibitors and MAP kinase inhibitors and Hsp90 inhibitors (including Geldanamycin), proteasome inhibitors (including Bortezomib) and HDAC inhibitors (including SAHA); prodrugs thereof.

[0121] The kit may also comprise instructions for use.

[0122] Examples embodying certain aspects of the invention will now be described with reference to the following figures in which:

[0123] FIG. **1** shows the 10 canonical (classical) pathways that were most over-represented in the signature list of PANC-1 cells in vitro treated with CXR1002 for 24 hrs relative to representation of these genes in the Ingenuity Database. (Accessed using Ingenuity Pathway Analysis (IPA) software available from Ingenuity Systems, Inc. (Redwood City Calif., USA)). P– values represent the likelihood that the association between the canonical pathways and the genes in the signature lists is due to random chance. The P-value is calculated with a right-tailed Fisher's Exact Test. The ratio represents the number of genes in a canonical pathway that are found in the signature lists divided by the total number of genes in the pathway.

[0124] FIG. **2** shows the changes in protein levels for PCNA (top) and cleaved PARP (bottom) in CXR1002-treated PANC-1 cells. PCNA is a marker for cell proliferation and cleaved PARP is representative of caspase cleavage and apoptosis. PANC-1 cells were exposed to CXR1002 at 450 μM concentration (Treated) for 24 hrs or DMSO vehicle (Control). Western blot analysis was performed with increasing amounts of protein, ranging between 2 and 20 μg (lanes 1-8). Positive control protein was derived from MCF7 cells (PCNA blot, lane 9) or from HeLa cells treated with staurosporine for 3 hours (Cleaved PARP blot, lanes 9, 10). Levels of total β-Actin are shown as a control for protein loading. Treated cells show increased cleaved PARP and reduced PCNA levels, indicating increased apoptosis and reduced proliferation respectively.

[0125] FIG. **3** shows the effects of CXR1002 on HT29 xenografts. Filled diamonds represent mean tumour volumes for animals treated with 25 mg/kg CXR1002 over time compared to those for saline treated control animals (empty squares). Tumour volumes were plotted using Graph Pad Prism software.

[0126] FIG. **4** shows the effects of CXR1002 on PC-3 xenografts. Filled diamonds represent mean tumour volumes for animals treated with 25 mg/kg CXR1002 over time compared to those for saline treated control animals (empty squares). Tumour volumes were plotted using Graph Pad Prism software.

[0127] FIG. **5** shows the effects of CXR1002 on PANC-1 tumours relative to the first day of treatment. Black line indicates the fold increase in tumour size for animals treated with 25 mg/kg CXR1002 over time compared to those for saline treated control animals (grey line).

[0128] FIG. **6** shows the effects of CXR1002 on PANC-1 tumour weights and tumour rigidity.

[0129] FIG. **7** shows the concentrations of CXR1002 in blood during the in-life stage of treatment, and in plasma and tumour tissue in terminal samples in treated (dark grey) versus control (light grey) animals.

[0130] FIG. **8** shows the effects of CXR1002 on HepG2 xenografts. Dark grey represents mean tumour volumes for animals treated with 25 mg/kg CXR1002 over time compared to those for saline treated control animals (light grey).

[0131] FIG. **9** shows the effects on tumour weight of HepG2 xenografts. Dark grey represents combined tumour weights for animals treated with 25 mg/kg CXR1002 over time compared to those for saline treated control animals (light grey).

[0132] FIG. **10** shows the plasma levels of CXR1002 over 6 weeks in a cohort of 3 patients after a single 50 mg dose.

[0133] FIG. **11** shows accumulating levels of CXR1002 following a repeat weekly 50 mg dose to for 6 weeks in a single patient.

[0134] FIG. **12** shows the increase in exposure with increasing dose level (50-450 mg) and duration (2-37 days) of a repeat weekly dose of CXR1002.

[0135] FIG. **13** shows a comparison of the exposure levels of PFOA in occupationally exposed workers compared to the exposure levels of CXR1002 in patients participating in the clinical trial.

[0136] FIG. **14** shows the average concentrations of APFO measured over 37 days for 3 patients dosed with a single dose of 50 mg of CXR1002.

[0137] FIG. **15** shows measured concentrations of APFO in patient 1 at 4 time points (days 144, 179, 227, 268) following a single dose of 50 mg of CXR1002.

[0138] FIG. **16** shows (a) accumulating levels of CXR1002 following a repeat weekly 100 mg dose for 6 weeks in patient 005 and (b) measured concentrations of APFO at 3 specific time points.

[0139] FIG. **17** shows accumulating levels of CXR1002 following a repeat weekly 100 mg dose for 6 weeks in patient 006.

[0140] FIG. **18** shows accumulating levels of CXR1002 following a repeat weekly 100 mg dose for 6 weeks in patient 007.

[0141] FIG. **19** shows accumulating levels of CXR1002 following a repeat weekly 200 mg dose for 6 weeks in patient 008.

[0142] FIG. **20** shows (a) accumulating levels of CXR1002 following a repeat weekly 200 mg dose for 6 weeks in patient 009 and (b) measured concentrations of CXR1002 at 3 specific time points.

[0143] FIG. **21** shows accumulating levels of CXR1002 following a repeat weekly 200 mg dose for 6 weeks in patient 010.

[0144] FIG. **22** shows accumulating levels of CXR1002 following a repeat weekly 300 mg dose for 6 weeks in patient 011.

[0145] FIG. **23** shows accumulating levels of CXR1002 following a repeat weekly 300 mg dose for 6 weeks in patient 012.

[0146] FIG. **24** shows accumulating levels of CXR1002 following a repeat weekly 450 mg dose for 6 weeks in patient 014.

[0147] FIG. **25** shows accumulating levels of CXR1002 following a repeat weekly 450 mg dose for 6 weeks in patient 015.

[0148] FIG. **26** shows accumulating levels of CXR1002 following a repeat weekly 450 mg dose for 6 weeks in patient 016.

[0149] FIG. **27** shows accumulating levels of CXR1002 following a repeat weekly 450 mg dose for 6 weeks in patient 017.

[0150] FIG. **28** shows a summary of the cytotoxicity assay results for test items combined with CXR1002 compared to treatment with test items alone. Medium grey (G)—more sensitive; light grey (y)—no change: dark grey (R)—possible decrease in sensitivity. Docetaxel when used alone in cytotoxicity assays gave unexpected results with most of the cell lines, as shown in the FIGS. **53-56**. The same results were obtained when the assays were repeated (data not shown). When used in combination with CXR1002, curves more usually associated with cytotoxicity assays were obtained (plotted as squares in graphs in FIGS. **53-56**).

[0151] FIG. **29** shows cytotoxicity plots for pancreatic cell lines treated with CXR1002 and Doxorubicin. Plots show percentage cell viability of cells treated in combination (squares) compared to Doxorubicin alone (triangles).

[0152] FIG. **30** shows cytotoxicity plots for ovarian cell lines treated with CXR1002 and Doxorubicin. Plots show percentage cell viability of cells treated in combination (squares) compared to Doxorubicin alone (triangles).

[0153] FIG. **31** shows cytotoxicity plots for sarcoma cell lines treated with CXR1002 and Doxorubicin. Plots show percentage cell viability of cells treated in combination (squares) compared to Doxorubicin alone (triangles).

[0154] FIG. **32** shows cytotoxicity plots for further cell lines treated with CXR1002 and Doxorubicin. Plots show percentage cell viability of cells treated in combination (squares) compared to Doxorubicin alone (triangles).

[0155] FIG. **33** shows cytotoxicity plots for pancreatic cell lines treated with CXR1002 and Gemcitabine. Plots show percentage cell viability of cells treated in combination (squares) compared to Gemcitabine alone (triangles).

[0156] FIG. **34** shows cytotoxicity plots for ovarian cell lines treated with CXR1002 and Gemcitabine. Plots show percentage cell viability of cells treated in combination (squares) compared to Gemcitabine alone (triangles).

[0157] FIG. **35** shows cytotoxicity plots for sarcoma cell lines treated with CXR1002 and Gemcitabine. Plots show percentage cell viability of cells treated in combination (squares) compared to Gemcitabine alone (triangles).

[0158] FIG. **36** shows cytotoxicity plots for further cell lines treated with CXR1002 and Gemcitabine, Plots show percentage cell viability of cells treated in combination (squares) compared to Gemcitabine alone (triangles).

[0159] FIG. **37** shows cytotoxicity plots for pancreatic cell lines treated with CXR1002 and Geldanamycin. Plots show percentage cell viability of cells treated in combination (squares) compared to Geldanamycin alone (triangles).

[0160] FIG. **38** shows cytotoxicity plots for ovarian cell lines treated with CXR1002 and Geldanamycin. Plots show percentage cell viability of cells treated in combination (squares) compared to Geldanamycin alone (triangles).

[0161] FIG. **39** shows cytotoxicity plots for sarcoma cell lines treated with CXR1002 and Geldanamycin. Plots show percentage cell viability of cells treated in combination (squares) compared to Geldanamycin alone (triangles).

[0162] FIG. **40** shows cytotoxicity plots for further cell lines treated with CXR1002 and Geldanamycin. Plots show percentage cell viability of cells treated in combination (squares) compared to Geldanamycin alone (triangles).

[0163] FIG. **41** shows cytotoxicity plots for pancreatic cell lines treated with CXR1002 and 5FU. Plots show percentage cell viability of cells treated in combination (squares) compared to 5FU alone (triangles).

[0164] FIG. **42** shows cytotoxicity plots for ovarian cell lines treated with CXR1002 and 5FU. Plots show percentage cell viability of cells treated in combination (squares) compared to 5FU alone (triangles).

[0165] FIG. **43** shows cytotoxicity plots for sarcoma cell lines treated with CXR1002 and 5FU. Plots show percentage cell viability of cells treated in combination (squares) compared to 5FU alone (triangles).

[0166] FIG. **44** shows cytotoxicity plots for further cell lines treated with CXR1002 and 5FU. Plots show percentage cell viability of cells treated in combination (squares) compared to 5FU alone (triangles).

[0167] FIG. **45** shows cytotoxicity plots for pancreatic cell lines treated with CXR1002 and Rapamycin. Plots show percentage cell viability of cells treated in combination (squares) compared to Rapamycin alone (triangles).

[0168] FIG. **46** shows cytotoxicity plots for ovarian cell lines treated with CXR1002 and Rapamycin. Plots show percentage cell viability of cells treated in combination (squares) compared to Rapamycin alone (triangles).

[0169] FIG. **47** shows cytotoxicity plots for sarcoma cell lines treated with CXR1002 and Rapamycin. Plots show percentage cell viability of cells treated in combination (squares) compared to Rapamycin alone (triangles).

[0170] FIG. **48** shows cytotoxicity plots for further cell lines treated with CXR1002 and Rapamycin. Plots show percentage cell viability of cells treated in combination (squares) compared to Rapamycin alone (triangles).

[0171] FIG. **49** shows cytotoxicity plots for pancreatic cell lines treated with CXR1002 and Roscovitine. Plots show percentage cell viability of cells treated in combination (squares) compared to Roscovitine alone (triangles).

[0172] FIG. **50** shows cytotoxicity plots for ovarian cell lines treated with CXR1002 and Roscovitine. Plots show percentage cell viability of cells treated in combination (squares) compared to Roscovitine alone (triangles).

[0173] FIG. **51** shows cytotoxicity plots for sarcoma cell lines treated with CXR1002 and Roscovitine. Plots show percentage cell viability of cells treated in combination (squares) compared to Roscovitine alone (triangles).

[0174] FIG. **52** shows cytotoxicity plots for further cell lines treated with CXR1002 and Roscovitine. Plots show percentage cell viability of cells treated in combination (squares) compared to Roscovitine alone (triangles).

[0175] FIG. **53** shows cytotoxicity plots for pancreatic cell lines treated with CXR1002 and Docetaxel. Plots show percentage cell viability of cells treated in combination (squares) compared to Docetaxel alone (triangles).

[0176] FIG. **54** shows cytotoxicity plots for ovarian cell lines treated with CXR1002 and Docetaxel. Plots show percentage cell viability of cells treated in combination (squares) compared to Docetaxel alone (triangles).

[0177] FIG. **55** shows cytotoxicity plots for sarcoma cell lines treated with CXR1002 and Docetaxel. Plots show percentage cell viability of cells treated in combination (squares) compared to Docetaxel alone (triangles).

[0178] FIG. **56** shows cytotoxicity plots for further cell lines treated with CXR1002 and Docetaxel. Plots show percentage cell viability of cells treated in combination (squares) compared to Docetaxel alone (triangles).

[0179] FIG. **57** shows cytotoxicity plots for pancreatic cell lines treated with CXR1002 and Cisplatin. Plots show percentage cell viability of cells treated in combination (squares) compared to Cisplatin alone (triangles).

[0180] FIG. **58** shows cytotoxicity plots for ovarian cell lines treated with CXR1002 and Cisplatin. Plots show percentage cell viability of cells treated in combination (squares) compared to Cisplatin alone (triangles).

[0181] FIG. **59** shows cytotoxicity plots for sarcoma cell lines treated with CXR1002 and Cisplatin. Plots show percentage cell viability of cells treated in combination (squares) compared to Cisplatin alone (triangles).

[0182] FIG. **60** shows cytotoxicity plots for further cell lines treated with CXR1002 and Cisplatin. Plots show percentage cell viability of cells treated in combination (squares) compared to Cisplatin alone (triangles).

[0183] FIG. **61** shows cytotoxicity plots for OMUS-27, H and SW1353 cells treated with CXR1002 alone (diamonds), in combination with UO126 (squares) and in combination with LY294002 (triangles).

[0184] FIG. **62** shows cytotoxicity plots for PANC1, BxPC3, HPAFII and Capan2 cells treated with CXR1002 alone (diamonds), in combination with UO126 (squares) and in combination with LY294002 (triangles).

[0185] FIG. **63** shows cytotoxicity plots for SK-OV3, TOV-21G, OV-90 and OVCAR3 cells treated with CXR1002 alone (diamonds) or in combination with UO126 (squares).

[0186] FIG. **64** shows a cytotoxicity plot Caco2 cells treated with CXR1002 alone (diamonds) or in combination with UO126 (squares).

[0187] FIG. **65** shows cytotoxicity plots for PANC-1, BxPc3, HPAFII and Capan2 cells treated with CXR1002 alone (diamonds) or in combination with DPQ (squares).

[0188] FIG. **66** shows cytotoxicity plots for OUMS-27, SW1353 and H cells treated with CXR1002 alone (diamonds) or in combination with DPQ (squares).

[0189] FIG. **67** shows accumulating levels of CXR1002 following a repeat weekly 600 mg dose for patient 18.

[0190] FIG. **68** shows accumulating levels of CXR1002 following a repeat weekly 600 mg dose for patient 20.

[0191] FIG. **69** shows accumulating levels of CXR1002 following a repeat weekly 600 mg does for patient 22.

[0192] FIG. **70** shows accumulating levels of CXR1002 following a repeat weekly 600 mg dose for patient 23.

[0193] FIG. **71** shows the effect of CXR1002 treatment or induction of expression of ER stress-regulated proteins. Lane designations are given in Example 8.

[0194] FIG. **72** shows splicing of XBPI mRNA induced in relation to CXR1002 induced ER stress.

[0195] FIG. **73** shows the percentage inhibition of PIM 1, PIM 2 and PIM 3 kinases as a dose response to CXR1002 exposure.

[0196] FIG. **74** shows CXR1002 plasma concentrations for a cohort of 6 patients after a repeat weekly 600 mg dose.

[0197] FIG. **75** shows the effects of dose increments on CXR1002 plasma exposure level over 6 weeks.

[0198] FIG. **76** shows the effects of dose increments on CXR1002 plasma exposure level over 6 weeks. Time points shown refer to pre-dose (TO) and thereafter (weekly) 24 hours post dose.

[0199] FIG. **77** shows the effect of dose increment on CXR1002 pharmacokinetics.

[0200] FIG. **78** shows the effect of dose increment on CXR1002 plasma exposure levels beyond the initial 6 week assessment period.

[0201] FIG. **79** shows the increase in urinary excretion of CXR1002 with duration of dosing.

[0202] FIG. **80** shows that the excretion of CXR1002 is reflected in the pharmacokinetic profile of a patient with high levels of urinary excretion.

[0203] FIG. **81** shows the effect of 6 weeks of CXR1002 treatment on plasma HDL-C levels.

[0204] FIG. **82** shows the effect of 6 weeks of CXR1002 treatment on plasma LDL-C levels.

[0205] FIG. **83** shows accumulating levels of CXR1002 following a repeat weekly 600 mg dose for 6 weeks in patient 024.

[0206] FIG. **84** shows accumulating levels of CXR1002 following a repeat weekly 600 mg dose for 6 weeks in patient 025.

[0207] FIG. **85** shows accumulating levels of CXR1002 following a repeat weekly 750 mg dose for 6 weeks in patient 026.

[0208] FIG. **86** shows accumulating levels of CXR1002 following a repeat weekly 750 mg dose for 6 weeks in patient 027.

[0209] FIG. **87** shows accumulating levels of CXR1002 following a repeat weekly 750 mg dose for 6 weeks in patient 028.

[0210] FIG. **88** shows accumulating levels of CXR1002 following a repeat weekly 950 mg dose for 6 weeks in patient 029.

[0211] FIG. **89** shows accumulating levels of CXR1002 following a repeat weekly 950 mg dose for 6 weeks in patient 030.

[0212] FIG. **90** shows accumulating levels of CXR1002 following a repeat weekly 950 mg dose for 6 weeks in patient 031.

[0213] FIG. **91** shows accumulating levels of CXR1002 following a repeat weekly 950 mg dose for 6 weeks in patient 032.

[0214] FIG. **93** shows accumulating levels of CXR1002 following a repeat weekly 1200 mg dose for 6 weeks in patient 033.

[0215] FIG. **94** shows accumulating levels of CXR1002 following a repeat weekly 1200 mg dose for 6 weeks in patient 034.

[0216] FIG. **94** shows accumulating levels of CXR1002 following a repeat weekly 1200 mg dose for 6 weeks in patient 035.

[0217] FIG. **95** shows accumulating levels of CXR1002 following a repeat weekly 1200 mg dose for 6 weeks in patient 036.

[0218] FIG. **96** shows accumulating levels of CXR1002 following a repeat weekly 1200 mg dose for 6 weeks in patient 037.

[0219] FIG. **97** shows accumulating levels of CXR1002 following a repeat weekly 1200 mg dose for 6 weeks in patient 038.

[0220] FIG. **98** shows accumulating levels of CXR1002 following a repeat weekly 1000 mg dose for 6 weeks in patient 040.

[0221] FIG. **99** shows accumulating levels of CXR1002 following a repeat weekly 1000 mg dose for 6 weeks in patient 041.

[0222] FIG. **100** shows accumulating levels of CXR1002 following a repeat weekly 1000 mg dose for 6 weeks in patient 042.

[0223] FIG. **101** shows accumulating levels of CXR1002 following a repeat weekly 600 mg dose for 6 weeks in patient 021.

PREFERRED EMBODIMENTS

Example 1

Induction of Peroxisome Proliferation

[0224] The earliest recognised characteristic of PPARα agonists was their ability to induce peroxisome proliferation in hepatocytes. The PPARα response is reflected in the increased transcription of mitochondrial and peroxisomal lipid metabolism, sterol, and bile acid biosynthesis and retinol metabolism genes (Andersen (2008)). Administration of APFO to rats led to hepatic peroxisome proliferation as measured by the induction of the peroxisomal marker activity cyanide-insensitive palmitoyl CoA oxidation (unpublished data).

[0225] Peroxisome proliferation occurs as a result of the interaction of a chemical with PPARα. This leads to an increase in the synthesis of peroxisomal and lipid-metabolising enzymes and, consequently, an increase in size and number of peroxisomes. Cyanide-insensitive palmitoyl CoA oxidation is an accepted marker of peroxisome proliferation, and was used to highlight PPARα activation in vitro and in vivo.

[0226] In vivo, APFO exhibits aspects of pharmacology typical of both PPARα and γ agonism. Male Sprague Dawley rats (n=6) were administered APFO (300 ppm) in powdered diet daily while control animals received powdered diet only. Rats were sacrificed at 7, 14, 28 and 84 days. Blood from each study animal was taken by cardiac puncture into lithium/heparin-coated tubes for separation of plasma. Plasma was analysed for glucose, triglycerides, cholesterol, AST and ALT (unpublished data).

[0227] Administration of APFO resulted in decreases in the plasma concentrations of triglycerides (PPARα-mediated) and glucose (PPARγ); plasma cholesterol levels were also reduced at all time points (Table 1). No adverse clinical observations were noted even after one year of continuous dietary dosing, although at early time points (1-2 weeks) slight elevations in plasma aspartate and alanine aminotransferase (AST and ALT) levels were observed. At this dietary dose level (300 ppm), plasma concentrations of APFO were 157.00±77.80 μM at week 2 and 256.96±38.93 μM at week 4.

TABLE 1

Effects of APFO on nutritional homeostasis in the rat. Data shown are mean ± standard deviation.

Nutritional parameters						
	Glucose		Triglycerides		Cholesterol	
Week	Control	APFO	Control	APFO	Control	APFO
1	19.00 ± 2.28	14.31 ± 1.88***	1.42 ± 0.40	0.41 ± 0.12***	2.18 ± 0.22	1.27 ± 0.41***
2	24.53 ± 5.53	16.98 ± 3.21**	1.58 ± 0.31	0.62 ± 0.13***	1.96 ± 0.34	1.55 ± 0.27**

TABLE 1-continued

Effects of APFO on nutritional homeostasis in the rat. Data shown are mean ± standard deviation.

4	25.19 ± 6.92	15.34 ± 2.64**	1.80 ± 0.79	0.57 ± 0.13***	2.30 ± 0.22	1.70 ± 0.29***
12	17.12 ± 2.36	13.12 ± 1.23***	1.69 ± 0.55	0.63 ± 0.15***	2.17 ± 0.26	1.75 ± 0.33*

Indicators of liver toxicity

	AST		ALT	
Days	Control	APFO	Control	APFO
1	107.80 ± 10.12	128.88 ± 6.21***	98.60 ± 9.01	101.90 ± 12.19
2	100.63 ± 4.63	120.00 ± 16.47**	81.60 ± 8.10	119.45 ± 19.27***
4	97.13 ± 13.56	101.80 ± 19.17	87.93 ± 11.54	98.99 ± 24.44
12	79.50 ± 9.86	92.63 ± 12.19*	71.67 ± 6.8	91.89 ± 14.88**

Statistical significance:
*$p \leq 0.05$;
**$p \leq 0.01$;
***$p \leq 0.001$

Interaction of CXR1002 with PPARs

[0228] Activation of PPARs is a transcriptional signature for PFOA in rats and mice, as well as common carp and zebrafish (Andersen (2008)). The effects of APFO and CXR1002 on the three PPAR isoforms in Cos-1 cells using a GAL4 binding assay and a transactivation assay using full length PPAR reporter gene constructs have been conducted using truncated PPAR constructs. The transactivation assay was performed in both agonist and antagonist mode (unpublished data). In antagonist mode for PPAR the finding from earlier assays suggesting reduced reporter expression, was confirmed by observation of direct antagonism activity for CXR1002. These findings are in keeping with those reported in independent studies by Vanden Heuvel et al., (2006) and Takacs & Abbott (2007), and are summarized together in Table 2.

Effects of CXR1002 on Other Nuclear Receptors

[0229] The effects of CXR1002 are not limited to PPARs. The non-selective pan-activation of numerous nuclear receptors is apparent not only by the transcriptional activation of many genes in PPARα-null mice (Rosen (2008)), but also by the scope of metabolic and regenerative pathways elicited by CXR1002 exposure. In particular, constitutive androstane receptor (CAR) and pregnenolone X receptor (PXR) are activated (Ren (2009)), although this appears to be on a species-specific basis. Further studies are needed, particularly on the human genes, to determine the significance of this in humans. Neither liver X receptor β (LXRβ) nor the common heterodimerization partner retinoid X receptor α (RXRα) are activated by PFOA (14).

TABLE 2

PPAR isoform agonism and antagonism reported using various assay systems.

	Dose CXR1002 or PFOA (μM)			
Assay	30	100	300	Reference
PPARα				
Human PPARα ligand binding	–	+	+	(12)
Human PPARα transactivation - full length in Cos-1 cells	–	–	++	(12)
Human PPARα transctivation - truncated in HEK293 T cells (agonist mode)	–	+	ND	(13)
Human PPARα transctivation - truncated in HEK293 T cells (antagonist mode using 10 μM ciprofibrate)	–	–	ND	(13)
Human PPARα transactivation in Cos-1 cells	+	ND	ND	(15)
Human PPARα transactivation in 3T3-L1 cells	ND	++	ND	(14)
PPARγ				
Human PPARγ ligand binding	–	–	++	(12)
Human PPARγ transactivation - full length in Cos-1 cells	–	–	+	(12)
Human PPARγ transctivation - truncated in HEK293 T cells (agonist mode)	–	–	–	(13)
Human PPARγ transctivation - truncated in HEK293 T cells (antagonist mode using 1 μM rosiglitazone)	–	–	+	(13)
Human PPARγ transactivation in Cos-1 cells	–	–	ND	(15)
Human PPARγ transactivation in 3T3-L1 cells	ND	–	ND	(14)
PPARδ				
Human PPARδ ligand binding	–	–	–	(12)
Human PPARδ transactivation - full length in Cos-1 cells	–	–	–	(12)
Human PPARδ transctivation - truncated in HEK293 T cells (agonist mode)	–	–	ND	(13)
Human PPARδ transctivation - truncated in HEK293 T cells (antagonist mode using 100 μM bezafibrate)	+	++	ND	(13)
Human PPARδ transactivation in Cos-1 cells	–	ND	ND	(15)

TABLE 2-continued

PPAR isoform agonism and antagonism reported using various assay systems.

	Dose CXR1002 or PFOA (μM)			
Assay	30	100	300	Reference
PPARα				
Human PPARα ligand binding	–	+	+	(12)
Human PPARα transactivation - full length in Cos-1 cells	–	–	++	(12)
Human PPARα transctivation - truncated in HEK293 T cells (agonist mode)	–	+	ND	(13)
Human PPARα transctivation - truncated in HEK293 T cells (antagonist mode using 10 μM ciprofibrate)	–	–	ND	(13)
Human PPARα transactivation in Cos-1 cells	+	ND	ND	(15)
Human PPARα transactivation in 3T3-L1 cells	ND	++	ND	(14)
PPARγ				
Human PPARγ ligand binding	–	–	++	(12)
Human PPARγ transactivation - full length in Cos-1 cells	–	–	+	(12)
Human PPARγ transctivation - truncated in HEK293 T cells (agonist mode)	–	–	–	(13)
Human PPARγ transctivation - truncated in HEK293 T cells (antagonist mode using 1 μM rosiglitazone)	–	–	+	(13)
Human PPARγ transactivation in Cos-1 cells	–	–	ND	(15)
Human PPARγ transactivation in 3T3-L1 cells	ND	–	ND	(14)
PPARδ				
Human PPARδ ligand binding	–	–	–	(12)
Human PPARδ transactivation - full length in Cos-1 cells	–	–	–	(12)
Human PPARδ transctivation - truncated in HEK293 T cells (agonist mode)	–	–	ND	(13)
Human PPARδ transctivation - truncated in HEK293 T cells (antagonist mode using 100 μM bezafibrate)	+	++	ND	(13)
Human PPARδ transactivation in Cos-1 cells	–	ND	ND	(15)

TABLE 2-continued

PPAR isoform agonism and antagonism reported using various assay systems.

Assay	Dose CXR1002 or PFOA (μM) 30	100	300	Reference
Human PPARδ transactivation in 3T3-L1 cells	ND	–	ND	(14)

ND = not done

Example 2

CXR1002 Induces ER Stress in Human Tumour Cells

[0230] To investigate the anti-tumour effects of CXR1002 in a non-biased manner, transcription profiling analysis was performed using the human pancreatic carcinoma cell line PANC-1 cultured in vitro. Gene expression changes observed in the normal pancreas are different from those in the liver, and suggest possible effects on gluconeogenesis and glutamine metabolism (Anderson (2008)). PANC-1 cells were treated with CXR1002 for 24 hrs at a concentration that has been found to cause 15% inhibition of cell growth (IC_{15}) and RNA was subsequently extracted. Analysis of the transcription profiles was made using pathways analysis in the Ingenuity system (unpublished data).

[0231] A list of 4996 genes was generated that showed changes in the treated samples compared to the untreated samples. Representation analysis of the in vitro 4996 signature list identified a number of pathways that were over-represented. In particular, genes in the endoplasmic reticulum (ER) stress pathway were over-represented in the signature list, FIG. **1**; Table 3. This included the ATF family of transcription factors (ATF3, ATF4 and ATF6) which are responsible for inducing ER stress and the unfolded protein response (UPR) (Szegezdi (2006)). ATF3 (induced-3 fold) was identified as a key transcription factor and pivotal component of the ER stress pathway.

[0232] The endoplasmic reticulum (ER) serves two major functions in the cell. It facilitates the proper folding of newly synthesised proteins destined for secretion and it provides the cell with a calcium reservoir. ER stress occurs in various physiological and pathological conditions where the capacity of the ER to fold proteins becomes saturated. Examples of these situations include calcium flux, glucose starvation, hypoxia or defective protein secretion, modification or degradation.

TABLE 3

Gene changes connected to ER stress in CXR1002-treated PANC-1 cells, as determined using Ingenuity Pathways Analysis software.

Symbol	Synonyms	Entrez Gene Name	Gene Function	Fold change	p-Value*
ATF4	C/ATF, CREB-2, MGC96460, TAXREB67, TXREB	Activating transcription factor 4 (tax-responsive enhancer element B67)	transcription regulator	1.818	2.47E−13
ATF6	ATF6 ALPHA, ATF6A, ESTM49	Activating transcription factor 6	transcription regulator	1.763	2.62E−10
CASP9	CASPASE-9, CASPASE-9c	Caspase 9, apoptosis-related cysteine peptidase	peptidase	1.291	2.40E−04
EIF2AK3	PERK, WRS	Eukaryotic translation initiation factor 2-alpha kinase 3	kinase	−1.266	4.62E−03
HSPA5	GRP78, HEAT SHOCK 70 KDA PROTEIN5	Heat shock 70 kDa protein 5 (glucose-regulated protein, 78 kDa)	other	2.414	7.23E−35
MAPK8	C-JUN N-TERMINAL KINASE1, JNK, JNK1	Mitogen-activated protein kinase 8	kinase	1.097	3.14E−03
MBTPS1	PCSK8	Membrane-bound transcription factor peptidase, site 1	peptidase	−1.170	7.60E−03
TAOK3	JIK, MAP3K18	TAO kinase 3	kinase	1.274	3.70E−04
XBP1	HTF, Sxbp-1, TREB-5, XBP2	X-box binding protein 1	transcription regulator	1.689	3.41E−10

*The p value calculated by Fishers test represents the probability that the association between genes in the signature list and the cannonical pathway (in this case ER stress) occurred by chance alone.

[0233] Cells respond to the accumulation of unfolded proteins in the ER by a rescue process called the unfolded protein response (UPR). However, if the unfolded protein accumulation is persistent and the stress cannot be relieved, UPR signalling switches from prosurvival to proapoptotic (Kim (2006)), (Szegezdi (2006)), usually involving processing of caspases (Chang (2006)). Consistent with this hypothesis, CXR1002-treated PANC-1 cells show reduced proliferation and cleavage of the caspase substrate poly-ADP ribose polymerase (PARP) (FIG. **2**) in PANC-1 cells.

[0234] Disruption of the UPR is particularly significant in certain tissues or organs, particularly those dedicated to extracellular protein synthesis e.g. glandular tissues such as the pancreas and thyroid. The pancreatic β-cell is particularly dependent on efficient UPR signalling due to the constantly varying demands for insulin synthesis (Marciniak (2006)).

[0235] Chemical toxicants such as tunicamycin and thapsigargin cause an accumulation of unfolded protein aggregates in the ER lumen (Schroder (2008)), (Harding (2002)), (Zhang (2008)). Whilst it is fair to say that many chemicals, drugs and toxicants induce ER stress, not all do. Microarray data from a previous unpublished study requires further analysis, but superficially at least seems to indicate that the ER stress effect may be specific to the pancreatic cancer cell line PANC-1 and not a feature of normal pancreas tissue since the ER stress response is not seen in normal pancreas treated with APFO (28 day study in rat). The phthalate DEHP and the PPARα agonist WY14,643 were also studied. No evidence of ER stress response was detected with either of these compounds.

[0236] In studies of primary rat hepatocytes, PFOA concentrations of 30 μM and above caused increased expression of DNA damage-inducible transcript 3 (DDIT3/CHOP/GADD153), suggesting ER stress (Bjork (2009)).

[0237] ER stress can be caused by the induction of oxidative enzymes and the CXR1002 PANC-1 microarray signatures showed some mRNA level induction of enzymes involved in redox homeostasis. Altered genes included glutamate-cysteine ligase modifier subunit (GCLM), glutamate-cysteine ligase catalytic subunit (GCLC), heme oxygenase (HO-1), glutathione reductase (GSR) and thioredoxin reductase (TRXR1) which are reflected by the over-representation of genes in the NRF2 signalling pathway. This is an indication that the PANG-1 cells are undergoing an oxidative stress response. The mechanism of this is unclear, however the induction of DNA damage response genes such as Growth arrest and DNA damage alpha (GADD45α), DDIT3, p21 and p53 suggest that oxidative stress may result in DNA damage. However, in follow-up experiments CXR1002 did not activate transcription of $p21^{WAF1}$, when examined using β-human chorionic gonadotrophin (hCG) excretion from reporter cell line A2780/$p21^{WAF1}$ exposed to CXR1002 for 24 hrs (unpublished data).

Discussion of Mechanism of Action/Target

[0238] The above data demonstrate that CXR1002 activates both PPARα and PPARγ at similar concentrations, potentially conferring the benefits of both receptors, including growth inhibition, induction of apoptosis and induction of terminal differentiation. Furthermore, CXR1002 may inhibit PPARδ. Given that PPARδ is able to oppose the effects of PPARα and PPARγ (Vosper (2001)) via repression of transcription mediated by competition for DNA binding (Shi (2002)), there may be a benefit to PPAR α/γ agonist which is inhibitory or neutral at the PPARδ receptor. CXR1002 may have effects on other nuclear receptors, such as CAR and PXR.

[0239] Induction of ER stress in tumour cells is a mechanistically important mode of action for a variety of anticancer drugs including bortezomib (Velcade) (Healy (2009)). It has also been shown to occur in mechanistic studies of PPAR agonists, such as the dual agonist thiazolidinedione TZD18 (Zang (2009)) and PPARγ ligands such as prostaglandin J2 (Weber 2004)), (Chamber (2007)). A direct correlation between ER stress and PPAR effect remains to be determined for CXR1002.

[0240] Overloading the UPR to induce cell death is a possible anticancer strategy (Healy (2009)). Recently, the UPR has been linked to hepatic lipid metabolism (Lee (2009)), and the finding that the transcription factor XBP1, best known as a key regulator of the UPR, is required for de novo fatty acid synthesis in the liver suggests this gene or gene pathway to be a key link (Lee (2008)).

Example 3

In Vitro Cytotoxicity of APFO and CXR1002

[0241] The Sulphorhodamine B (SRB) assay was used to determine the in vitro cytotoxicity of APFO (CXR1001) and CXR1002 towards a panel of human tumour-derived cell lines in a 48 hr assay. The SRB assay was performed according to the method specified by the NIC/NIH. The results for ten cell lines using the SRB assay are summarised in Table 4. The lowest IC_{50} values (~160 μM) were seen with HepG2 cells and the highest (~740 μM) were seen with CaCo-2 cells. In every case the cytotoxic effects of APFO and CXR1002 were similar. In subsequent experiments with CXR1002 an ATP cytotoxicity assay was used on a panel of 18 tumour cell lines. The effects of CXR1002 were assessed after 48 hr treatment. Assay replicates were independent in time and up to 4 replicates were performed per cell line. In this study, some cell lines were resistant to CXR1002, or produced dose response curves which did not allow for IC_{50} determination. A 48 hr assay may not produce optimal cytotoxicity; recent data shows that a 7 day endpoint gives lower cytotoxicity IC_{50} values (data not shown).

TABLE 4

In vitro cytotoxicity of APFO and CXR1002 using the SRB assay (48 hrs).

Cell		IC_{50} Values (μM) After 48 Hrs Exposure	
Line	Tissue Type	APFO (CXR1001)	CXR1002
HT-29	Colon Adenocarcinoma	283.3 ± 5.8	341.7 ± 10.6
CaCo-2	Colon Adenocarcinoma	736.7 ± 244.8	708.3 ± 159.1
MCF-7	Mammary Adenocarcinoma	388.3 ± 227.3	275.7 ± 33.2
MDA-MB-157	Mammary Carcinoma	511.7 ± 194.5	463.3 ± 167.7
HepG2	Hepatoblastoma	167.3 ± 97.1	161.7 ± 31.8
Hep3B	Hepatocarcinoma	273.3 ± 80.2	215.0 ± 28.3
PC3	Prostate Adenocarcinoma	316.7 ± 59.2	271.7 ± 40.7
A549	Non-Small Cell Lung Carcinoma	223.3 ± 35.4	230.0 ± 21.8
A2780	Ovarian Carcinoma	260.0 ± 28.3	237.7 ± 7.5
A375	Malignant Melanoma	230.0 ± 10.0	221.7 ± 21.2

TABLE 5

In vitro cytotoxicity of CXR1002 using the ATP depletion cytotoxicity assay (48 hrs).

Cell Line	Tissue type	IC_{50} 1	IC_{50} 2	IC_{50} 3	IC_{50} 4	Mean	SD
H	Chondrosarcoma	550	555	800		635.00	142.92
OUMS-27	Chondrosarcoma	1000	1000	800	800*	900.00	115.47
SW1353	Chondrosarcoma	>1000	>1000	>1000	>1000*	>1000	
PANC-1	Pancreatic Epithelioid carcinoma	>1000	>1000	>1000	880**	>1000	
BxPc3	Pancreatic Adenocarcinoma	370	350	400		373.33	25.17
HPAFII	Pancreatic Adenocarcinoma	>1000	>1000	750		>1000	
Capan2	Pancreatic Adenocarcinoma	850	850	750		816.67	57.74
SK-OV3	Ovarian Adenocarcinoma	550	535	520		535.00	15.00
TOV-21G	Ovarian Adenocarcinoma	610	600	700		636.67	55.08
OV-90	Ovarian Adenocarcinoma	650	650	670		656.67	11.55
OVCAR-3	Ovarian Adenocarcinoma	650	650			650.00	0.00
PC3	Prostate Adenocarcinoma	650	650	625		641.67	14.43
CaCo-2	Colon Adenocarcinoma	680	690	670		680.00	10.00
MDA-MB-157	Mammary medullary tumour	670	675	650		665.00	13.23
HepG2	Hepatoblastoma	240	230	350		273.33	66.58
U2OS	Osteosarcoma	>1000*					
MES-SA	Uterine sarcoma	>1000*					
HT1080	Fibrosarcoma	>1000*					
Canine Hepatocytes	Hepatocyte	240*					

*Study CXR0798;
**Study CXR0786;
All other data: Study CXR0859

[0242] The mechanism of cytotoxicity of APFO and CXR1002 was evaluated using bromodeoxyuridine (BrdU) incorporation to quantify cell proliferation and Hoechst 3342 staining to identify apoptotic cells. Significant suppression of BrdU incorporation was observed in all but one of the cell lines used in the SRB cytotoxicity assay following treatment with 300 μM APFO or CXR1002 for 48 hrs; in five cell lines, no proliferating cells were detectable at this concentration. No marked effects were observed at 10 μM, whereas the response to 30 μM was variable. The concentration dependence of induction of apoptosis was similar, with marked induction of apoptosis at 300 μM, little effect at 10 μM and variable responses at 30 μM.

Example 4

In Vivo Activity of CXR1002

[0243] CXR1002 has been examined in a small number of xenograft models, using both intra-peritoneal (i.p) and oral dosing (p.o). The effect of PFOA on HT-29 (colon adenocarcinoma) tumours was assessed in nude mouse xenografts, initially using APFO and subsequently using CXR1002. Animals were inoculated with a tumour cell suspension on each flank and the tumours were allowed to grow for 16 days. CXR1002 was administered intra-peritoneally three times per week for 28 days; results were graphed using a curve-fitting programme (FIG. **3**). At 25 mg/kg, CXR1002 had an anti-tumour effect on HT-29 tumour volumes. No significant compound-dependent effects on body weight were detected (results not shown), but an increase in liver weight (up to 2.5 fold) was observed. The maximum plasma concentration of CXR1002 detected was 277 μM following this dosing regimen.

[0244] A parallel experiment was carried out using the prostate tumour cell line PC3. Xenograft tumours derived from PC3 cells grew much more slowly than HT-29 xenografts; nevertheless, CXR1002 had a marked anti-tumour effect in this model. The effects of different doses of CXR1002 (5, 15 and 25 mg/kg given by the i.p route) were very similar in this experiment, but for simplification, only data from the 25 mg/kg group is shown (FIG. **4**). No marked effects on body weight were detected, but again an increase in liver weight was observed. The maximum plasma concentration of CXR1002 detected was 281 μM in mice treated with 25 mg/kg three times weekly.

[0245] In both the HT-29 and PC-3 xenograft experiments, slight reductions in plasma glucose and triglyceride levels were detected following CXR1002 treatment of tumour-bearing nude mice, consistent with activation of the PPARγ and PPARα receptors, respectively. Slight increases (up to 3.5 fold) in plasma AST occurred in response to CXR1002 in mice bearing either HT-29 or PC3 cell xenografts. Plasma ALT levels were only slightly increased in PC3-tumour bearing mice (up to 1.8 fold) and were actually decreased in mice bearing HT-29 xenografts. These effects are consistent with a transient effect on the liver associated with mild toxicity and

reversible liver enlargement. In rodents, this type of effect is usually due to hepatic PPARα activation associated with peroxisome proliferation.

[0246] A further xenograft model was performed using the human pancreatic cell line PANC-1. This tumour is slow growing in vivo. Female nude mice were implanted with PANG-1 cells and once the tumours reached a pre-determined size the animals were dosed with CXR1002 at 25 mg/kg, 3 times per week. For various reasons, animals were lost during the study and the final group sizes were small. Nevertheless, the CXR1002 treated animals showed substantially delayed tumour growth and the weights and rigidity of the tumours were also different between the vehicle treated and untreated animals (FIG. **5**, FIG. **6**). This experiment is currently being repeated to try to obtain larger group sizes at experimental completion.

[0247] In-life and terminal blood samples taken from the mice were analysed for CXR1002 levels using a validated analytical method. In-life samples averaged 146 μM and terminal blood samples (24 hours post final dose) averaged 474 μM (FIG. **7**). Plasma values were higher than the whole blood values. This may be attributed to the duration of dosing. In addition, CXR1002 is highly plasma protein bound. Furthermore, the erythrocyte/plasma partitioning coefficient (which measures the amount of drug bound to red cells compared to plasma binding) may contribute to the observed differences.

[0248] CXR1002 was also tested in a xenograft model of liver carcinoma using the cell line HepG2. In this experiment CXR1002 was dosed at 25 mg/kg in two different regimens: 2× per week and 3× per week. Although this tumour cell line is particularly sensitive to CXR1002 in vitro, the xenografted tumours showed a modest response in terms of growth inhibition. There was no obvious difference between the two different dosing regimens. The data in FIG. **8** and FIG. **9** shows the combined data from the 2 different treatment dosing regimens for tumour growth and tumour weight, respectively. The terminal plasma concentrations of CXR1002 were 437 μM for the 2× weekly regimen and 520 μM for the 3× weekly regimen.

[0249] To summarise, CXR1002 has been tested in four human tumour xenograft models, HT-29 (colon), PC3 (prostate), PANC-1 (pancreatic) and HepG2 (liver). Anti-tumour effects were detected in all models as shown in Table 6. No significant toxicity was observed, although there was evidence for minor changes in liver enzyme function, associated with a liver enlargement effect, which is probably rodent-specific. The exposure to CXR1002 in nude mice was lower than the blood levels achieved in patients at the higher doses in the CXR1002-001 phase I trial.

TABLE 6

Summary of best response in xenograft models

Cell Line and Tumour Model	Absolute Tumour Volume as a Percentage of Saline Control*	Day of evaluation	Terminal CXR1002 plasma levels (collected 24 hrs post last dose)	In vitro 48 hr IC_{50} for CXR1002
HT-29 (Colon)	49.22%	Day 29	277 μM (Day 29)	341.7 ± 10.6**
PC3 (Prostate)	19.14%	Day 25	281 μM (Day 29)	641.67 ± 14.43#
HepG2 (Liver)	77.05%	Day 53	437 ± 127 μM (×2 weekly regimen) 520 ± 46 μM (×3 weekly regimen) (Days 60-64)	273.33 ± 66.58#
PANC-1 (Pancreas)	50.13%	Day 82	474 ± 153 μM (Day 88)	>1000#

*Saline control = 100%
**Data from Table 4
#Data from Table 5

Other Relevant Pharmacology

[0250] PPARs play key roles in nutritional homeostasis, the primary effects of PPARα being in the regulation of fatty acid catabolism and those of PPARγ being in adipose differentiation and insulin-mediated regulation of glucose levels (2), (3). The hypolipidaemic effects of PPARα agonists are well characterised, while more recent studies have demonstrated the hypoglycaemic effects of PPARγ agonists (47), (48), (49), (50). While these effects may be peripheral to the anticancer effects of CXR1002, they are relevant as hypotriglyceridaemia and hypoglycaemia may be used as pharmacodynamic markers of PPAR α and γ agonism respectively.

Example 5

Human Clinical Data

[0251] CXR1002 monotherapy has been evaluated in a single Phase I trial in cancer patients with the primary objective of determining the maximum tolerated dose (MTD) of a weekly dosing schedule. A summary of this trial is provided in Table 7.

TABLE 7

Clinical Trial of CXR1002

Protocol Number	Study Design	Dosing Schedule	Dose Levels	Status
CXR1002-001	Phase I	Weekly dose	single dose: 50 mg repeat dose: 50 mg,	Ongoing (n = 43)

TABLE 7-continued

Clinical Trial of CXR1002

Protocol Number	Study Design	Dosing Schedule	Dose Levels	Status
			100 mg, 200 mg, 300 mg, 450 mg, 600 mg, 750 mg, 950 mg, 1000 mg, 1200 mg	

CXR 1002 was administered in powder-filled hard gelatin capsules. One dose-strength oral capsules was used (50 mg).

[0252] The bulk active pharmaceutical ingredient will be manufactured under GMP conditions by Chimete Srl, Italy; and the capsules manufactured to cGMP by Penn Pharmaceutical Services LTD, UK.

[0253] Storage: All trial medication was held in a dry place at room temperature (15° C. to 25° C.) and protected from light.

[0254] The starting dose of CXR1002 was 50 mg administered orally as a single dose. This is approximately 0.24× the Lowest Observed Effect dose level in the monkey which is the most sensitive species that was tested.

[0255] CXR1002 was administered to patients, as a capsule by the oral route, orally as a single dose of 50 mg in the morning after an overnight fast in the first cohort of 3 patients. Prophylactic anti-emetics were not administered, and patients fasted for 1 hour after ingestion of CXR1002. PK samples, PD (fasting) samples, blood glucose, and blood triglyceride samples, were taken over a 6-week period.

[0256] These patients then underwent repeat dosing schedule with the same dose of CXR1002. The repeat dosing schedule was weekly administration of CXR1002 as a single oral dose in the morning and patients fasted for 1 hour before and after ingestion of CXR1002. Dose limiting toxicity (DLT) will be based on the toxicity assessments over the first 3-week period of the repeat dosing schedule. PK samples (single blood sample) were taken on the following basis:

[0257] Every 6 weeks during the repeat dosing phase

[0258] If dosing is interrupted or stopped, samples will be taken at intervals according to patient convenience

[0259] PK sampling for safety evaluation may take place at any time, as clinically indicated

[0260] In all dose cohorts subsequent to the initial dose cohort, all patients will be treated with weekly administration of study drug from the start of dosing. Dose escalation was performed after all patients at the preceding dose level had completed a 3-week repeat dosing period. The dose of CXR1002 was increased in successive dose cohorts until ≧Grade 2 drug-related toxicity was observed, after which dose escalation was in approximately 30% increments.

[0261] As of February, 2011, 43 patients with advanced cancers from one Phase I study have received CXR1002. The weekly dose administered ranges from 50 to 1200 mg.

[0262] The best response to CXR1002 treatment was stable disease by investigator assessment. One patient with pancreatic cancer had stable disease lasting 7 months.

[0263] Pharmacokinetic analysis of CXR1002 was carried out in the Phase I study using a validated assay. After oral administration of a single dose of CXR1002, the plasma concentration reached a Cmax at 1.5 hours in all 3 patients examined. After a single 50 mg dose the exposure in 3 patients varied between 8 and 16 μM and this was maintained at a constant level over the 6 week sampling period following the dose. The data indicates the half-life of elimination of CXR1002 could not be defined but is >6 weeks.

[0264] After weekly repeat doses of CXR1002 the plasma level increased in stepped increments. The maximal plasma level recorded to date was from a patient who had received a 1200 mg weekly dose over a 5 week period and had a plasma level of 1530 μM.

[0265] There appeared to be no gender difference in CXR1002 exposure following CXR1002 administration. The drug is eliminated extremely slowly and accumulates following a weekly dose.

[0266] Study CXR1002-001 is an open label, two centre, phase I study in patients with advanced cancer to assess the tolerability, safety and pharmacokinetics of CXR1002 administered weekly. The study synopsis is shown in Table 8.

TABLE 8

Study Synopsis for Study CXR1002-001 (n = 43)

	Study Synopsis
Design	Open label
Study Period	First Patient In: 02Sep08 Last Patient Out: To be determined Study period: Approximately 3 years
Study Drug	CXR1002 50 mg capsule
Objectives	Primary: To determine the safety, toxicity and dose limiting toxicity (DLT) and maximum tolerated dose (MTD) of CXR1002 when given as single oral dose on a weekly schedule. Secondary: To describe the pharmacokinetics; to investigate the effect of CXR1002 on markers of PPAR agonist activity; to assess anti-tumour activity; to propose a safe dose for phase II.
Patient Population	Male and female patients with advanced solid tumours that are refractory to standard therapy or for which no standard therapy exists.
Number of Patients (planned and treated)	Planned: Up to 50 Treated: 43
Drug Administration	Oral
Dosing	Single 50 mg dose Weekly dose; dose escalation starting at 50 mg
Dose Escalation	Up to 100% dose escalation permitted
Treatment Duration	6 weeks with pharmacokinetic sampling, thereafter as long as patient receives a benefit
MTD	To be determined

[0267] Forty three patients were enrolled in the study, as of February 2011. Thirty two patients were enrolled at the Beatson West of Scotland Cancer Centre, Glasgow, and eleven patients were enrolled at Aberdeen Royal Infirmary.

[0268] CXR1002 is being given orally as a weekly dose. The starting dose was a 50 mg single dose. The starting weekly repeat dose was 50 mg, with 2 patients continuing to the repeat dose schedule after receiving a single dose. Doses were escalated in groups of three patients. The dose escalation is continuing. A summary of the dose escalation is provided in Table 9.

TABLE 9

Dose Escalation Summary (n = 43)

Dose (mg/week)	Number of Patients
50	4 (1 patient received only single dose)
100	3
200	3
300	4
450	3
600	7
750	3
950	4
1000	5
1200	6

[0269] A validated analytical assay consisting of non-GLP LC-MS/MS was used to quantitate CXR1002 in human plasma. Plasma samples were collected after the single 50 mg dose at the following timepoints: Pre-dose, and then 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 24, 48, and 72 hours after administration and then once weekly at weeks 2, 3, 4, 5, and 6 (days 8, 15, 22, 29, and 36). For patients treated with the weekly repeat dose, plasma samples were collected at the following timepoints: Pre-dose and then 2, 3, 4 and 24 hours after administration for a total of 6 weeks. Thereafter a single sample was collected every 6 weeks for monitoring of exposure during long term treatment. Plasma samples were processed at site and stored at −80° C. prior to batch shipment to the analytical laboratory.

[0270] Of the 43 patients enrolled in study CXR1002-001, 24 were males and 19 were females. The majority of patients had received 2 prior therapies. Two patients had received 5 prior therapies. The tumour types of the patients are shown in Table 10.

TABLE 10

Patient demographics: Tumour type on Study CXR1002-001 (n = 43)

Cancer Type	Number of Patients
pancreatic	6
parathyroid	1
gastric	1
renal	2
colorectal	17
breast	1
cervix	1
anaplastic thyroid	1
vulval	1
lung	1
oesophageal	3
unknown primary	3
carcinoid	1
melanoma	2
sarcoma	1
nasopharyngeal carcinoma	1

[0271] Pharmacodynamic samples were also collected from patients for the measurement of pharmacodynamic markers. Samples were collected using the same time schedule as that used for the pharmacokinetic samples.

Pharmacokinetic Sample Analysis

[0272] The following data shows for each patient the plasma levels over time. The particular weekly dose is shown, as is the gender and age of each patient. Graphical plots of the data for each patient are shown in FIGS. **10** to **27**, **67** to **70** and **83** to **101**.

TABLE 11

(a-an)

(a) Patient 001
Date of Birth: 07.07.1944
Dose: 50 mg
Sex: Male

Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)
0	0.00	24	13.81	Day 227	307.9
0.25	0.35	48	12.76	Day 268	268.0
0.5	1.11	72	9.70		
0.75	9.17	192 (Day 8)	8.54		
1	14.41	360 (Day 15)	8.63		
1.5	25.72	528 (Day 22)	11.58		
2	22.48	696 (Day 29)	10.23		
3	20.82	864 (Day 36)	8.89		
4	18.19	Day 144	226.2		
6	14.67	Day 179	250.5		

(b) Patient 002
Date of Birth: 21.05.1950
Dose: 50 mg
Sex: Female

Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)
0	0.00	6	17.52
0.25	3.06	24	14.27
0.5	7.62	48	13.28
0.75	8.39	72	15.60
1	8.55	192 (Day 8)	17.15
1.5	29.79	360 (Day 15)	18.61
2	24.07	528 (Day 22)	21.47
3	22.76	696 (Day 29)	20.96
4	14.45	864 (Day 36)	20.08

(c) Patient 003
Date of Birth: 29.12.19[illegible]
Dose: 50 mg
Sex: Male

Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)
0	0.14	6	16.90
0.25	1.08	24	19.53
0.5	11.30	48	18.07
0.75	17.05	72	18.43
1	20.69	192 (Day 8)	8.60
1.5	24.64	360 (Day 15)	7.20
2	24.49	528 (Day 22)	6.50
3	21.15	696 (Day 29)	5.00
4	17.98	864 (Day 36)	6.50

TABLE 11-continued

(a-an)

(d) Patient 004
Date of Birth: 15.09.1954
Dose: 50 mg
Sex: Female

Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)
0	0.10	336 (Day 15)	21.81	672 (Day 29)	52.28
2	19.95	338	31.09	674	65.78
3	16.94	339	38.75	675	61.74
4	19.26	340	40.60	676	56.69
24 (Day 2)	14.39	360 (Day 16)	38.75	696 (Day 30)	77.49
168 (Day 8)	12.76	504 (Day 22)	33.37	840 (Day 36)	63.04
170	35.73	506	46.29	842	78.63
171	40.37	507	50.41	843	80.55
172	35.96	508	47.88	844	No sample
192 (Day 9)	22.51	528 (Day 23)	49.96	864 (Day 37)	81.07

(e) Patient 005
Date of Birth: 27.02.1941
Dose: 100 mg
Sex: Male

Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)
0	0.23	338	80.20	675	100.84
2	19.83	339	70.09	676	82.74
3	23.01	340	53.92	696 (Day 30)	78.80
4	23.66	360 (Day 16)	47.40	840 (Day 36)	99.43
24 (Day 2)	19.94	504 (Day 22)	48.39	842	79.99
168 (Day 8)	18.96	506	84.00	843	98.91
170	42.88	507	74.40	844	91.44
171	50.82	508	87.35	864 (Day 37)	109.10
172	45.71	528 (Day 23)	57.87	Day 92	276.33
192 (Day 9)	34.37	672 (Day 29)	80.77		
336 (Day 15)	47.82	674	99.65		

(f) Patient 006
Date of Birth: 12.04.1943
Dose: 100 mg
Sex: Male

Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)
0	0.21	336 (Day 15)	33.10	672 (Day 29)	86.94
2	32.32	338	49.50	674	85.73
3	27.07	339	55.91	675	n.s
4	24.62	340	n.s	676	n.s
24 (Day 2)	28.18	360 (Day 16)	65.25	696 (Day 30)	89.54
168 (Day 8)	26.16	504 (Day 22)	70.55	840 (Day 36)	85.61
170	36.65	506	76.44	842	179.07
171	41.15	507	83.83	843	137.87
172	47.47	508	n.s	844	138.38

TABLE 11-continued

(a-an)

192 (Day 9)	44.66	528 (Day 23)	97.00	864 (Day 37)	122.64

(g) Patient 007
Date of Birth: 06.01.1963
Dose: 100 mg
Sex: Female

Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)
0	0.00	336 (Day 15)	19.23	672 (Day 29)	
2	30.91	338	52.84	674	
3	28.55	339	53.14	675	
4	19.26	340	55.78	676	
24 (Day 2)	17.29	360 (Day 16)	32.01	696 (Day 30)	
168 (Day 8)	12.23	504 (Day 22)	39.69	840 (Day 36)	
170	n.s	506	53.12	842	
171	n.s	507	65.79	843	
172	n.s	508	73.03	844	
192 (Day 9)	n.s	528 (Day 23)	63.42	864 (Day 37)	

(h) Patient 008
Date of Birth: 21.01.1940
Dose: 200 mg
Sex: Male

Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)
0	0.00	336 (Day 15)	124.19	672 (Day 29)	337.46
2	114.25	338	147.04	674	355.90
3	102.46	339	172.18	675	321.30
4	81.02	340	191.50	676	364.45
24 (Day 2)	70.37	360 (Day 16)	210.97	696 (Day 30)	426.16
168 (Day 8)	64.56	504 (Day 22)	276.84	840 (Day 36)	424.66
170	171.02	506	282.15	842	360.53
171	142.88	507	368.27	843	396.83
172	129.91	508	280.95	844	414.33
192 (Day 9)	110.01	528 (Day 23)	284.86	864 (Day 37)	354.39

(i) Patient 009
Date of Birth: 11.03.1973
Dose: 200 mg
Sex: Female

Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)
0	0.00	338	161.42	675	261.46
2	93.43	339	167.24	676	358.20
3	61.21	340	152.60	696 (Day 30)	338.09
4	71.13	360 (Day 16)	218.26	840 (Day 36)	399.10
24 (Day 2)	64.58	504 (Day 22)	253.19	842	345.61
168 (Day 8)	60.06	506	285.24	843	373.31
170	148.65	507	295.84	844	329.34
171	170.29	508	362.32	864 (Day 37)	346.17
172	138.60	528 (Day 23)	241.69	Day 92	456.38

TABLE 11-continued

(a-an)

192 (Day 9)	136.45	672 (Day 29)	251.16	Week 19	617.56
336 (Day 15)	127.41	674	471.59	Week 25	500.93
				Week 32	540.20

(j) Patient 010
Date of Birth: 29.01.1959
Dose: 200 mg
Sex: Male

Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)
0	0.00	336 (Day 15)	98.79	672 (Day 29)	192.34
2	21.07	338	118.05	674	192.67
3	30.35	339	103.02	675	236.98
4	33.61	340	66.68	676	230.90
24 (Day 2)	58.60	360 (Day 16)	174.52	696 (Day 30)	256.06
168 (Day 8)	51.00	504 (Day 22)	181.86	840 (Day 36)	242.97
170	81.72	506	207.19	842	188.17
171	81.39	507	276.15	843	232.44
172	119.44	508	186.77	844	222.78
192 (Day 9)	101.39	528 (Day 23)	190.86	864 (Day 37)	220.65

(k) Patient 011
Date of Birth: 15.04.1961
Dose: 300 mg
Sex: Male

Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)
0	n.d	336 (Day 15)	131.32	672 (Day 29)	254.90
2	100.88	338	159.58	674	291.09
3	107.29	339	160.00	675	304.27
4	111.65	340	146.91	676	242.69
24 (Day 2)	95.87	360 (Day 16)	237.26	696 (Day 30)	326.13
168 (Day 8)	57.87	504 (Day 22)	145.38	840 (Day 36)	269.65
170	112.25	506	203.57	842	384.08
171	122.41	507	288.21	843	350.35
172	138.33	508	243.62	844	282.27
192 (Day 9)	178.42	528 (Day 23)	241.39	864 (Day 37)	386.77
				Week 12	573.70

(l) Patient 012
Date of Birth: 19.06.1945
Dose: 300 mg
Sex: Male

Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)
0	n.d	336 (Day 15)	141.25	672 (Day 29)	303.06
2	91.38	338	240.93	674	340.42
3	93.53	339	196.29	675	372.99
4	122.90	340	213.09	676	291.97
24 (Day 2)	74.05	360 (Day 16)	218.10	696 (Day 30)	334.20
168 (Day 8)	64.83	504 (Day 22)	180.56	840 (Day 36)	
170	182.32	506	277.77	842	
171	147.52	507	260.84	843	

TABLE 11-continued

(a-an)

172	126.45	508	221.25	844	
192 (Day 9)	151.74	528 (Day 23)	263.16	864 (Day 37)	

(m) Patient 013
Date of Birth: 04.09.1957
Dose: 300 mg
Sex: Female

Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)
0	n.d	336 (Day 15)		672 (Day 29)	
2	82.72	338		674	
3	75.97	339		675	
4	85.32	340		676	
24 (Day 2)	79.61	360 (Day 16)		696 (Day 30)	
168 (Day 8)		504 (Day 22)		840 (Day 36)	
170		506		842	
171		507		843	
172		508		844	
192 (Day 9)		528 (Day 23)		864 (Day 37)	

(n) Patient 014
Date of Birth: 01.09.1937
Dose: 300 mg
Sex: Male

Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)
0	n.d.	336 (Day 15)	173.32	672 (Day 29)	354.70
2	131.24	338	297.35	674	389.10
3	120.77	339	270.41	675	399.93
4	100.33	340	261.42	676	411.42
24 (Day 2)	101.72	360 (Day 16)	n.s	696 (Day 30)	478.38
168 (Day 8)	79.64	504 (Day 22)	287.79	840 (Day 36)	425.93
170	154.12	506	335.79	842	506.31
171	178.41	507	373.83	843	520.08
172	179.97	508	n.s	844	562.63
192 (Day 9)	178.39	528 (Day 23)	420.49	864 (Day 37)	487.60
				Week 12	889.60
				Week 18	979.62

(o) Patient 015
Date of Birth: 26.06.1939
Dose: 450 mg
Sex: Female

Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)
0	n.d.	336 (Day 15)	286.27	672 (Day 29)	543.57
2	176.20	338	463.43	674	622.36
3	231.36	339	393.32	675	632.84
4	216.70	340	424.29	676	675.19
24 (Day 2)	126.15	360 (Day 16)	411.72	696 (Day 30)	707.80
168 (Day 8)	137.59	504 (Day 22)	386.72	840 (Day 36)	575.19
170	324.96	506	578.86	842	702.79
171	314.27	507	548.01	843	655.16
172	n.s.	508	513.31	844	800.55

TABLE 11-continued

(a-an)

192 (Day 9)	294.21	528 (Day 23)	550.55	864 (Day 37) Week 13	592.16 1174.82

(p) Patient 016
Date of Birth: 11.11.1957
Dose: 450 mg
Sex: Female

Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)
0	n.d.	336 (Day 15)	286.80	672 (Day 29)	605.12
2	107.59	338	417.75	674	906.59
3	125.90	339	454.11	675	745.49
4	88.14	340	442.28	676	732.34
24 (Day 2)	164.05	360 (Day 16)	470.53	696 (Day 30)	871.19
168 (Day 8)	181.57	504 (Day 22)	545.74	840 (Day 36)	
170	327.77	506	693.48	842	
171	255.06	507	721.48	843	
172	243.61	508	697.87	844	
192 (Day 9)	348.41	528 (Day 23)	692.36	864 (Day 37)	

(q) Patient 017
Date of Birth: 09.12.1933
Dose: 450 mg
Sex: Female

Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)
0	n.d.	336 (Day 15)	216.41	672 (Day 29)	403.72
2	99.68	338	325.48	674	451.12
3	136.14	339	341.96	675	497.22
4	163.18	340	No sample	676	484.61
24 (Day 2)	127.31	360 (Day 16)	258.49	696 (Day 30)	483.20
168 (Day 8)	146.02	504 (Day 22)	332.56	840 (Day 36)	434.00
170	276.16	506	427.08	842	460.92
171	252.67	507	411.89	843	456.72
172	248.08	508	No sample	844	482.23
192 (Day 9)	245.77	528 (Day 23)	405.28	864 (Day 37)	525.98

(r) Patient 018
Date of Birth: 23.05.1940
Dose: 600 mg
Sex: Female

Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)
0	n.d.	336 (Day 15)	356.38	672 (Day 29)	
2	338.52	338	551.28	674	
3	280.28	339	563.34	675	
4	248.26	340	590.95	676	
24 (Day 2)	213.23	360 (Day 16)	553.60	696 (Day 30)	
168 (Day 8)	180.97	504 (Day 22)		840 (Day 36)	
170	397.91	506		842	
171	406.73	507		843	
172	n.s.	508		844	

TABLE 11-continued

(a-an)

192 (day 9)	385.85	528 (Day 23)		864 (Day 37)

(s) Patient 020
Date of Birth: 20.04.1966
Dose: 600 mg
Sex: Male

Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)
0	n.d.	336 (Day 15)	271.93	672 (Day 29)	540.35
2	207.66	338	474.01	674	547.27
3	182.13	339	433.87	675	603.48
4	179.81	340	437.8	676	651.85
24 (Day 2)	413.39	360 (Day 16)	410.21	696 (Day 30)	615.23
168 (Day 8)	125.29	504 (Day 22)	440.64	840 (Day 36)	582.17
170	327.38	506	477.40	842	700.19
171	309.51	507	526.45	843	764.41
172	278.89	508	536.35	844	770.32
192 (day 9)	268.45	528 (Day 23)	562.88	864 (Day 37)	702.75

(t) Patient 021
Date of Birth: 28.08.1958
Dose: 600 mg
Sex: Female

Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)
0	n.d.	336 (Day 15)	412.22	672 (Day 29)	613.22
2	77.23	338	649.84	674	681.46
3	120.90	339	652.79	675	847.13
4	117.32	340	633.0	676	845.20
24 (Day 2)	203.29	360 (Day 16)	604.18	696 (Day 30)	799.22
168 (Day 8)	217.79	504 (Day 22)	604.54	840 (Day 36)	778.84
170	504.50	506	734.36	842	968.41
171	416.60	507	654.13	843	927.77
172	450.70	508	721.72	844	980.72
192 (Day 9)	478.75	528 (Day 23)	677.90	864 (Day 37)	995.39

(u) Patient 022
Date of Birth: 11.05.1959
Dose: 600 mg
Sex: Male

Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)
0	n.d.	336 (Day 15)	289.10	672 (Day 29)	
2	192.81	338	433.41	674	
3	196.01	339	433.35	675	
4	198.74	340	405.3	676	
24 (Day 2)	156.54	360 (Day 16)	427.22	696 (Day 30)	
168 (Day 8)	143.49	504 (Day 22)	426.00	840 (Day 36)	
170	309.80	506	441.08	842	
171	269.82	507	561.63	843	
172	275.79	508	518.62	844	
192 (day 9)	298.41	528 (Day 23)	595.95	864 (Day 37)	

TABLE 11-continued

(a-an)

(v) Patient 023
Date of Birth: 10.06.1940
Dose: 600 mg
Sex: Male

Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)
0	n.d.	336 (Day 15)	431.55	672 (Day 29)	
2	208.10	338	494.51	674	
3	233.12	339	613.01	675	
4	236.13	340	635.73	676	
24 (Day 2)	221.19	360 (Day 16)	616.06	696 (Day 30)	
168 (Day 8)	204.11	504 (Day 22)		840 (Day 36)	
170	335.70	506		842	
171	336.75	507		843	
172	390.84	508		844	
192 (day 9)	400.07	528 (Day 23)		864 (Day 37)	

(w) Patient 024
Date of Birth: 14.09.1939
Dose: 600 mg
Sex: Female

Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)
0	n.d.	336 (Day 15)	488.31	672 (Day 29)	788.40
2	91.59	338	577.60	674	693.74
3	187.52	339	532.72	675	779.35
4	282.55	340	628.20	676	780.28
24 (Day 2)	257.81	360 (Day 16)	647.35	696 (Day 30)	810.90
168 (Day 8)	211.59	504 (Day 22)	691.46	840 (Day 36)	813.92
170	435.55	506	642.15	842	897.68
171	440.70	507	705.87	843	903.71
172	439.24	508	630.45	844	908.35
192 (Day 9)	418.38	528 (Day 23)	858.92	864 (Day 37)	966.13

(y) Patient 026
Date of Birth: 23.07.1951
Dose: 750 mg
Sex: Male

Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)
0	n.d.	336 (Day 15)	352.35	672 (Day 29)	593.90
2	95.82	338	399.42	674	605.61
3	124.60	339	505.65	675	655.20
4	149.03	340	492.30	676	697.97
24 (Day 2)	200.07	360 (Day 16)	624.63	696 (Day 30)	732.46
168 (Day 8)	185.64	504 (Day 22)	478.99	840 (Day 36)	728.08
170	270.45	506	625.39	842	823.68
171	301.45	507	546.33	843	775.85
172	339.13	508	581.68	844	821.51
192 (Day 9)	397.76	528 (Day 23)	607.97	864 (Day 37)	811.72

TABLE 11-continued

(a-an)

(z) Patient 027
Date of Birth: 17.10.1943
Dose: 750 mg
Sex: Female

Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)
0	n.d.	336 (Day 15)	365.66	672 (Day 29)	605.65
2	109.52	338	384.07	674	768.97
3	240.51	339	411.73	675	811.16
4	224.48	340	451.09	676	756.91
24 (Day 2)	199.39	360 (Day 16)	569.22	696 (Day 30)	803.44
168 (Day 8)	167.86	504 (Day 22)	476.33	840 (Day 36)	672.92
170	235.24	506	658.10	842	
171	317.57	507	668.76	843	
172	344.46	508	671.26	844	
192 (Day 9)	410.69	528 (Day 23)	719.70	864 (Day 37)	

(aa) Patient 028
Date of Birth: 29.07.1944
Dose: 750 mg
Sex: Male

Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)
0	n.d.	336 (Day 15)	288.21	672 (Day 29)	521.82
2	206.86	338	472.99	674	709.85
3	173.85	339	395.89	675	713.40
4	160.39	340	405.8	676	700.01
24 (Day 2)	159.66	360 (Day 16)	450.13	696 (Day 30)	757.67
168 (Day 8)	160.74	504 (Day 22)	467.30	840 (Day 36)	682.49
170	321.26	506	581.50	842	824.29
171	301.29	507	489.55	843	791.81
172	272.96	508	581.26	844	853.05
192 (Day 9)	295.12	528 (Day 23)	654.60	864 (Day 37)	774.65

(ab) Patient 029
Date of Birth: 19.05.1946
Dose: 950 mg
Sex: Female

Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)
0	n.d.	336 (Day 15)	438.90	672 (Day 29)	492.32
2	218.65	338	703.63	674	841.18
3	250.85	339	777.16	675	971.71
4	307.26	340	673.88	676	812.35
24 (Day 2)	352.58	360 (Day 16)	896.30	696 (Day 30)	949.34
168 (Day 8)	288.92	504 (Day 22)	400.09	840 (Day 36)	777.26
170	603.87	506	896.90	842	792.81
171	601.54	507	741.47	843	n.s.
172	556.45	508	700.77	844	n.s.
192 (Day 9)	606.03	528 (Day 23)	781.22	864 (Day 37)	1043.20

TABLE 11-continued

(a-an)

(ac) Patient 030
Date of Birth: 19.12.1944
Dose: 950 mg
Sex: Male

Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)
0	n.d.	336		672	
		(Day 15)		(Day 29)	
2	116.56	338		674	
3	219.04	339		675	
4	332.61	340		676	
24	214.26	360		696	
(Day 2)		(Day 16)		(Day 30)	
168		504		840	
(Day 8)		(Day 22)		(Day 36)	
170		506		842	
171		507		843	
172		508		844	
192		528		864	
(Day 9)		(Day 23)		(Day 37)	

(ad) Patient 031
Date of Birth: 13.11.1952
Dose: 950 mg
Sex: Male

Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)
0	n.d.	336	554.28	672	812.71
		(Day 15)		(Day 29)	
2	266.82	338	668.41	674	920.63
3	337.51	339	715.03	675	882.04
4	335.80	340	697.4	676	938.32
24	347.52	360	799.77	696	1031.14
(Day 2)		(Day 16)		(Day 30)	
168	237.01	504	754.63	840	
(Day 8)		(Day 22)		(Day 36)	
170	512.93	506	794.18	842	
171	463.15	507	861.48	843	
172	426.87	508	998.35	844	
192	515.81	528	958.09	864	
(Day 9)		(Day 23)		(Day 37)	

(ae) Patient 032
Date of Birth: 17.09.1935
Dose: 950 mg
Sex: Male

Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)
0	BLQ	336		672	
		(Day 15)		(Day 29)	
2	291.69	338		674	
3	191.19	339		675	
4	231.38	340		676	
24	160.60	360		696	
(Day 2)		(Day 16)		(Day 30)	
168	192.28	504		840	
(Day 8)		(Day 22)		(Day 36)	
170	439.06	506		842	
171	478.03	507		843	
172	516.70	508		844	
192	495.07	528		864	
(Day 9)		(Day 23)		(Day 37)	

TABLE 11-continued

(a-an)

(af) Patient 033
Date of Birth: 19.08.1936
Dose: 1200 mg
Sex: Male

Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)
0	0.00	336	637.00	672	1019.28
		(Day 15)		(Day 29)	
2	441.43	338	925.60	674	1231.36
3	395.64	339	840.74	675	1112.56
4	373.92	340	816.70	676	1096.93
24	371.41	360	915.01	696	1105.61
(Day 2)		(Day 16)		(Day 30)	
168	317.41	504	713.28	840	1040.57
(Day 8)		(Day 22)		(Day 36)	
170	734.84	506	1147.58	842	1114.49
171	657.48	507	1061.41	843	1226.74
172	637.11	508	1007.50	844	1204.86
192	590.63	528	1172.58	864	1317.84
(Day 9)		(Day 23)		(Day 37)	

(ag) Patient 034
Date of Birth: 15.04.1948
Dose: 1200 mg
Sex: Female

Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)
0	n.d	336	644.35	672	1079.09
		(Day 15)		(Day 29)	
2	440.36	338	1026.11	674	1308.93
3	401.05	339	1115.82	675	1448.79
4	559.64	340	954.51	676	1383.21
24	409.45	360	1024.33	696	1307.12
(Day 2)		(Day 16)		(Day 30)	
168	350.51	504	923.50	840	
(Day 8)		(Day 22)		(Day 36)	
170	893.14	506	1354.14	842	
171	770.66	507	1217.57	843	
172	729.99	508	1440.82	844	
192	721.75	528	1363.80	864	
(Day 9)		(Day 23)		(Day 37)	

(ah) Patient 035
Date of Birth: 28.08.1958
Dose: 1200 mg
Sex: Male

Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)
0	n.d	336	476.33	672	
		(Day 15)		(Day 29)	
2	298.06	338	570.40	674	
3	196.36	339	621.24	675	
4	316.74	340	649.3	676	
24	261.19	360	704.40	696	
(Day 2)		(Day 16)		(Day 30)	
168	254.36	504	599.62	840	
(Day 8)		(Day 22)		(Day 36)	
170	459.98	506	774.20	842	
171	478.99	507	925.12	843	
172	592.29	508	1022.64	844	
192	542.94	528	1172.95	864	
(Day 9)		(Day 23)		(Day 37)	

TABLE 11-continued

(a-an)

(al) Patient 040
Date of Birth: 20.06.1952
Dose: 1000 mg
Sex: Male

Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)
0	n.d.	336 (Day 15)	305.35	672 (Day 29)	489.94
2	187.66	338	487.42	674	558.12
3	167.62	339	426.74	675	532.65
4	189.71	340	476.48	676	607.14
24 (Day 2)	148.06	360	484.91	696 (Day 30)	697.26
168 (Day 8)	119.66	504 (Day 22)	353.16	840 (Day 36)	573.56
170	367.81	506	501.95	842	625.73
171	343.25	507	554.18	843	747.73
172	325.68	508	515.30	844	No sample
192 (Day 9)	272.17	528 (Day 23)	524.78	864 (Day 37)	826.44

(am) Patient 041
Date of Birth: 23.05.1945
Dose: 1000 mg
Sex: Male

Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)
0	n.d.	336 (Day 15)	294.39	672 (Day 29)	670.13
2	222.94	338	411.42	674	546.09
3	232.54	339	485.07	675	739.71
4	207.41	340	478.48	676	802.50
24 (Day 2)	175.01	360 (Day 16)	508.44	696 (Day 30)	785.67
168 (Day 8)	171.34	504 (Day 22)	558.23	840 (Day 36)	714.83
170	342.33	506	674.45	842	737.74
171	412.52	507	748.03	843	1006.29
172	357.46	508	676.27	844	No sample
192 (Day 9)	395.56	528 (Day 23)	703.40	864 (Day 37)	1209.31

(an) Patient 042
Date of Birth: 21.02.1947
Dose: 1000 mg
Sex: Female

Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)
0	n.d.	336 (Day 15)	585.96	672 (Day 29)	1036.40
2	271.56	338	645.49	674	1178.80
3	358.73	339	675.08	675	1191.64
4	281.49	340	636.03	676	No sample
24 (Day 2)	238.71	360 (Day 16)	650.70	696 (Day 30)	1281.13
168 (Day 8)	250.60	504 (Day 22)	764.91	840 (Day 36)	1248.6
170	391.37	506	871.78	842	1140.5
171	461.28	507	951.85	843	1177.0
172	435.82	508	986.97	844	1158.6
192 (Day 9)	501.59	528 (Day 23)	1231.51	864 (Day 37)	1251.9

TABLE 11-continued

(a-an)

(ai) Patient 036
Date of Birth: 23.03.1946
Dose: 1200 mg
Sex: Female

Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)
0	n.d.	336 (Day 15)	556.91	672 (Day 29)	No sample
2	442.58	338	840.43	674	No sample
3	431.11	339	865.85	675	No sample
4	360.54	340	930.0	676	No sample
24 (Day 2)	708.42	360 (Day 16)	657.31	696 (Day 30)	No sample
168 (Day 8)	285.95	504 (Day 22)	968.95	840 (Day 36)	947.50
170	679.68	506	1143.19	842	1247.49
171	678.01	507	1131.52	843	1293.03
172	665.00	508	1117.46	844	1234.17
192 (Day 9)	295.33	528 (Day 23)	No sample	864 (Day 37)	1063.33

(aj) Patient 037
Date of Birth: 19.04.1958
Dose: 1200 mg
Sex: Female

Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)
0	n.d.	336 (Day 15)	779.78	672 (Day 29)	1316.96
2	255.87	338	1053.68	674	1457.82
3	302.40	339	1044.19	675	1456.41
4	278.36	340	1025.26	676	1451.35
24 (Day 2)	418.44	360 (Day 16)	1135.41	696 (Day 30)	1530.33
168 (Day 8)	422.38	504 (Day 22)	1104.32	840 (Day 36)	
170	633.83	506	1199.98	842	
171	659.95	507	1223.32	843	
172	711.66	508	1393.91	844	
192 (Day 9)	841.24	528 (Day 23)	1387.00	864 (Day 37)	

(ak) Patient 038
Date of Birth: 06.10.1957
Dose: 1200 mg
Sex: Female

Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)	Time (hr)	Concentration (μM)
0	n.d.	336 (Day 15)	461.60	672 (Day 29)	681.90
2	312.62	338	808.36	674	931.50
3	314.43	339	682.38	675	884.53
4	297.98	340	672.23	676	708.47
24 (Day 2)	241.95	360 (Day 16)	749.49	696 (Day 30)	802.27
168 (Day 8)	268.04	504 (Day 22)	566.42	840 (Day 36)	687.10
170	502.09	506	658.21	842	895.36
171	480.58	507	787.75	843	954.75
172	538.47	508	698.94	844	913.67
192 (Day 9)	492.87	528 (Day 23)	746.12	864 (Day 37)	958.10

n.s----No sample;

Pharmacokinetic Summary

[0273] The half life of CXR1002 is extremely long and could not be defined during the period of evaluation (6 weeks) (FIG. **10**). CXR1002 accumulates in the blood following each weekly dose. This is exemplified in FIG. **11**, which shows accumulating plasma levels after 6 weekly 50 mg doses in patient 01-004. There is greater exposure with increasing dose of CXR1002 and with increasing duration of treatment (FIG. **12**). The maximal blood level reached was 617 μM.

Efficacy

[0274] The best response to CXR1002 treatment was stable disease lasting 7 months. Four patients had stable disease ≧4 months (range 20 to 35 weeks) (Table 12). Of these, 1 patient diagnosed with pancreatic cancer had radiographic evidence of tumour shrinkage which did not meet the criteria of partial response.

TABLE 12

Patients with Stable Disease (SD) >4 months on Study CXR1002-001

Dose (mg/week)	Tumour Type	Best response	On-Study Duration (weeks)	Number Prior Treatments
50	pancreatic	SD	35	2
100	colorectal	SD	20	3
200	cervical	SD	22*	2
200	anaplastic thyroid	SD	22*	3

*Patient remains on study

Example 6

Combinations of CXR1002 with Other Drugs

[0275] The aim of this study was to combine CXR1002 with other agents to ascertain whether an enhanced response to the combination of drugs was observed.

[0276] The results as presented are from a single assay in which the cell lines listed in Table 13 below were exposed to CXR1002 or the test items listed in Table 14 or the test items in combination with CXR1002.

[0277] FIG. **28** shows a tabulated summary of the results taken from the individual graphs of the cytotoxicity assays on individual cell lines 2 (black curve—test item alone; blue curve—test item+a single dose of CXR1002). Green indicates that the cells were more sensitive to a combination of test item and CXR1002 than to the test item alone. Yellow indicates that there was no apparent change in sensitivity and therefore no further analyses is suggested. Red indicates a possible adverse effect of the combination of drug with CXR1002. The full data is shown in FIGS. **29-60**.

Methods

[0278] The cell lines were purchased from The American Type Culture Collection (ATCC) via LGC Promochem (London, UK), the European Collection of Cell Cultures (ECACC) via Sigma-Aldrich, UK, or the Health Science Research Resources Bank of the Japan Health Science Foundation (JHSF): (Refer to Table 13). Cell line H was supplied by the Biomedical Research Centre, Ninewells Hospital, Dundee.

TABLE 13

Cell lines purchased from commercial suppliers and stored at CXR Biosciences:

Cell Line	Tissue	Supplier	Product Code
OUMS-27	Sarcoma	JHSF	IFO50488
SW1353	Sarcoma	ATCC	HTB-94
PANC1	Pancreas	ATCC	CRL-1469
BxPc3	Pancreas	ATCC	CRL-1687
HPAFII	Pancreas	ATCC	CRL-1997
Capan2	Pancreas	ATCC	HTB-80
SK-OV3	Ovary	ATCC	HTB-77
TOV-21G	Ovary	ATCC	CRL-11730
OV-90	Ovary	ATCC	CRL-11732
OVCAR3	Ovary	ATCC	HTB-161
PC3	Prostate	ECACC	90112714
MDA-MB-157	Breast	ECACC	92020422
CACO2	Colon	ECACC	86010202
HepG2	Liver	ECACC	85011430

TABLE 14

Test Item Supplier Details

Test Item	Supplier	Catalogue Ref.
Doxorubicin hydrochloride	Sigma	D1515
Gemcitabine	Sequoia	SRP01265g
Cisplatin	Sigma	P4394
Docetaxel	Fluka	01885
5-FU	Sigma	F6627
Roscovitine	Sigma	R7772
DPQ	Sigma	D5314
Geldanamycin	Apollo scientific	BIG2461
Rapamycin	Apollo scientific	BIR8101
LY294002	Sigma	L9908
U0126	Merck	662005

[0279] Test compounds were dissolved in DMSO to make stock solutions of an appropriate concentration. The stock solutions were further diluted in DMSO to produce additional stock solutions as necessary. The amount of DMSO added to the medium was 1% of the final volume.

[0280] Cells were plated at the optimal plating density for that cell line in 96-well plates and allowed to attach overnight. The next day, the medium was removed and replaced with fresh medium containing the dose ranges of test items. The cells were exposed to 5-FU, cisplatin, docetaxel, doxorubicin, geldanamycin, gemcitabine, rapamycin or roscovitine in Roswell Park Memorial Institute (RPMI) medium containing 10% Foetal Calf Serum (FCS) and 2 mM Glutamine at 37° C. and 5% CO_2 for 48 hours. The concentrations of CXR1002 or other agents to which the cells were exposed were as previously determined or as suggested by relevant literature (see Table 14 below). There were 3 replicates for each test item concentration.

TABLE 15

Final concentrations of compounds in tissue culture medium.

Compound	Final Concentration μM			
5-Fluorouracil	1	10	100	300
Cisplatin	1	10	100	300
CXR1002	100	300	500	1000
Docetaxel	0.1	1	10	100
Doxorubicin	0.01	0.1	1	10
Geldanamycin	0.0001	0.001	0.1	1

TABLE 15-continued

Final concentrations of compounds in tissue culture medium.

Compound	Final Concentration μM			
Gemcitabine	1	10	100	300
Rapamycin	0.0001	0.001	0.1	1
Roscovitine	1	3	10	50

[0281] From the results of the single compound assays, appropriate doses ranges were determined for use in the combinatorial assays with CXR 1002. These are shown in Table 16:

[0282] Following exposure to Test Items, the CellTitre-Glo Luminescent Cell Viability Assay to measure ATP content was performed according to the manufacturer's detailed instructions (Promega Corporation, Technical Bulletin No. 288, and Cell notes, Issue 10, 2004).

[0283] The results of the ATP depletion assay were corrected for background luminescence and expressed as a percentage of the vehicle control value using Microsoft Excel software. Point-to-point spline analysis was performed and the results graphed in GraphPad Prism as cell viability (percentage of vehicle control) versus Test Item concentration.

TABLE 16

Final concentrations of compounds for cytotoxicity assays in combination with CXR1002.

Cell Line	[1002]μM	[Doxorubicin]μM				[Gemcitabine]μM				[Cisplatin]μM			
H	300	0.001	0.01	0.1	1	0.01	0.1	1	100	0.1	1	10	30
OUMS-27	300	0.01	0.1	0.5	5	0.01	0.1	1	100	1	3	5	10
SW1353	300	0.01	0.1	0.2	0.5	1	10	100	300	10	20	30	50
PANC1	300	0.01	0.1	0.5	1	0.01	0.1	1	100	1	10	30	50
BxPc3	150	0.01	0.1	0.5	1	0.01	0.1	1	100	1	10	20	30
HPAFII	300	0.01	0.1	0.5	5	0.01	0.1	1	100	10	20	50	100
Capan2	300	0.1	1	3	5	0.1	1	10	100	10	100	150	200
SK-OV3	300	0.001	0.01	0.1	1	0.001	0.01	0.1	1	1	10	30	50
TOV-21G	300	0.01	0.05	0.1	0.25	0.01	0.1	1	50	1	10	15	30
OV-90	300	0.1	0.3	1	10	0.1	1	10	100	10	30	100	300
OVCAR-3	300	0.01	0.03	0.1	0.3	0.01	0.1	1	50	0.1	1	10	20
PC3	300	0.01	0.1	1	10	0.1	1	10	100	1	10	100	300
CACO2	300	0.1	0.5	1	5	0.1	1	10	100	10	20	30	50
MDA-MB-157	300	0.1	0.3	0.5	2	1	10	100	300	10	100	200	300
HepG2	100	0.01	0.1	0.2	0.5	0.01	0.1	1	10	10	20	30	50

Cell Line	[1002]μM	[Docetaxel]μM				[5FU]μM				[Roscovitine]μM			
H	300	0.01	0.1	1	10	1	10	100	300	3	10	20	30
OUMS-27	300	0.01	0.1	1	10	1	10	100	300	3	10	20	30
SW1353	300	0.001	0.01	0.1	1	1	10	100	300	1	5	10	50
PANC1	300	0.001	0.01	0.1	1	1	10	100	300	1	5	10	50
BxPc3	150	0.001	0.01	0.1	1	1	10	100	300	1	5	10	20
HPAFII	300	0.01	0.1	1	100	1	10	100	300	1	5	10	50
Capan2	300	0.1	1	10	100	1	10	100	300	3	10	30	50
SK-OV3	300	0.001	0.01	0.1	1	1	10	100	300	3	10	30	50
TOV-21G	300	0.01	0.1	1	10	1	10	100	300	1	5	10	20
OV-90	300	0.01	0.1	1	100	1	10	100	300	3	10	30	50
OVCAR-3	300	0.01	0.1	1	10	0.1	1	10	100	1	3	10	20
PC3	300	0.1	1	10	100	1	10	100	300	1	3	10	50
CACO2	300	0.01	0.1	1	10	1	10	100	300	1	3	10	30
MDA-MB-157	300	0.001	0.01	0.1	1	1	10	100	300	1	3	10	30
HepG2	100	0.001	0.01	0.1	1	1	10	100	300	1	3	10	30

	[1002]μM	[Geldanamycin]μM				[Rapamycin]μM			
H	300	0.001	0.01	0.1	0.5	0.001	0.1	1	10
OUMS-27	300	0.001	0.01	0.1	0.5	0.001	0.1	1	10
SW1353	300	0.001	0.01	0.1	1	0.001	0.1	1	10
PANC1	300	0.1	1	5	10	0.001	0.1	1	10
BxPc3	150	0.001	0.01	0.1	0.5	0.0001	0.001	0.01	0.1
HPAFII	300	0.1	1	5	10	0.001	0.1	1	10
Capan2	300	0.1	1	5	10	0.001	0.1	1	10
SK-OV3	300	0.1	1	5	10	0.001	0.1	1	10
TOV-21G	300	0.001	0.01	0.1	1	0.0001	0.001	0.01	0.1
OV-90	300	0.001	0.01	0.1	1	0.001	0.1	1	10
OVCAR-3	300	0.001	0.01	0.1	1	0.0001	0.001	0.01	0.1
PC3	300	0.1	1	5	10	0.0001	0.001	0.01	0.1
CACO2	300	0.001	0.01	0.1	1	0.0001	0.001	0.01	0.1
MDA-MB-157	300	0.1	1	5	10	0.0001	0.001	0.01	0.1
HepG2	100	0.001	0.01	0.1	0.5	0.001	0.1	1	10

Notes:

HepG2 and BxPc3 cells are more sensitive to treatment with CXR1002 than the other lines used in these assays. As a result the single dose of CXR1002 used for HepG2 cells in the combination assays as 100 μM and for BxPc3 cells this was 150 μM.

Conclusions

[0284] Doxorubicin, gemcitabine, geldanamycin and roscovitine were shown to increase the sensitivity of a number of the cell lines. Rapamycin increased sensitivity in the four ovarian lines tested and in HepG2 cells. Interestingly from the clinical perspective, when MDA-MB-157 (breast) cells were treated with the combination of 5-FU, a drug used in the treatment of breast cancer, and CXR1002, there was an apparent increase in sensitivity.

TABLE 17

Summary of Conclusions

Cell Line	Tissue	Drugs for repeat cytoxicity assays in combination with CXR1002
Panc1	Pancreas	Doxorubicin, Gemcitabine, Geldanamycin
BxPc3	Pancreas	Gemcitabine
Capan2	Pancreas	Roscovitine
H	Chondrosarcoma	Gemcitabine
SK-OV3	Ovary	Rapamycin
TOV-21G	Ovary	Gemcitabine, 5-FU
OV-90	Ovary	Doxorubicin, Geldanamycin, Rapamycin, Roscovitine
OVCAR-3	Ovary	Gemcitabine
PC3	Prostate	Doxorubicin, Geldanamycin, Roscovitine
MDA-MB-157	Breast	5-FU
HepG2	Liver	Gemcitabine, Geldanamycin, Rapamycin, Roscovitine

Example 7

Further Combination Data

[0285] The objective of this study is to combine CXR1002 with known anti-cancer agents, both investigational and marketed drugs, in an effort to achieve enhanced tumour cell killing ie. to potentiate mode of action.

[0286] In a 48 hour cytotoxicity assay the following compounds were tested at fixed concentrations, derived from a review of the literature, in the presence of CXR1002 (0-1 mM):

1. MAP kinase inhibitor (MEK1/2) (compound name, UO126)
2. AKT/PI3K inhibitor (compound name, LY294002)
3. PARP inhibitor (compound name, DPQ)

TABLE 18

Cell signalling inhibitors used in this study

Chemical	Indication	Concentration (literature)	Concentration used in this study	Duration	Mechanism	Ref.
U0126	Breast cancer cell lines	i) 6, 12.5, or 50 μM ii) IC_{50}: 10-20 μM	10 μM	Up to 10 h, activity may decline after longer incubation	MEK1/2 inhibitor	Mol. Cancer Ther., 303-309, (2002)
LY294002	Pancreatic cell lines (+cisplatin)	10-75 μM IC_{50}: 50 μM, 10-25 μM At 50 μM no toxic effect	12.5 μM		Akt/PI3K inhibitor	J. Exp. & Clin. Res., (2008)
DPQ		20-30 μM	20 μM		PARP inhibitor	Anticancer Drug Designs., 107 (1991)

Method

[0287] The cell lines were purchased from The American Type Culture Collection (ATCC) via LGC Promochem (London, UK), the European Collection of Cell Cultures (ECACC) via Sigma-Aldrich, UK, or the Health Science Research Resources Bank of the Japan Health Science Foundation (JHSF): (Refer to Table 19). Sarcoma cell line H was supplied by the Biomedical Research Centre, Ninewells Hospital, Dundee.

TABLE 19

Cell lines purchased from commercial suppliers and stored at CXR Biosciences:

Cell Line	Tissue	Supplier	Product Code
OUMS-27	Sarcoma	JHSF	IFO50488
SW1353	Sarcoma	ATCC	HTB-94
PANC-1	Pancreas	ATCC	CRL-1469
BxPc3	Pancreas	ATCC	CRL-1687
HPAFII	Pancreas	ATCC	CRL-1997
Capan2	Pancreas	ATCC	HTB-80
SK-OV3	Ovary	ATCC	HTB-77
TOV-21G	Ovary	ATCC	CRL-11730
OV-90	Ovary	ATCC	CRL-11732
OVCAR3	Ovary	ATCC	HTB-161
PC3	Prostate	ECACC	90112714
MDA-MB-157	Breast	ECACC	92020422
CACO2	Colon	ECACC	86010202
HepG2	Liver	ECACC	85011430

TABLE 20

Test Item Supplier Details

Test Item	Supplier	Catalogue Ref.
U0126	Sigma	U0126
LY294002	Sigma	L9908
DPQ	Sigma	D5314

Cell Culture

[0288] Test compounds were dissolved in DMSO to make stock solutions of an appropriate concentration. The stock solutions were further diluted in DMSO to produce additional stock solutions as necessary. The concentrations of the origi-

nal stock solutions and the additional stock solutions will be recorded in the appropriate CXR Study folder and in the Study Report. The final amount of DMSO added to the medium was 1% of the final volume.

[0289] Cells were plated at the optimal plating density for that cell line in 96-well plates and allowed to attach overnight. The next day, cells were pre-treated with the inhibitors UO126 or LY294002 (see Tables 18 & 20) for 2 hrs, the medium was removed and replaced with fresh medium containing the appropriate dose of the test item. After 2 hrs, CXR1002 (concentration range 0-1 mM) together with the appropriate inhibitor was then added. Cells that were to be treated with DPQ received no pre-treatment. Cells were exposed to these compounds in Roswell Park Memorial Institute (RPMI) medium containing 10% Foetal Calf Serum (FCS) and 2 mM Glutamine at 37° C. and 5% CO_2 for 48 hours. There were 3 replicates for each test item concentration.

[0290] Following exposure to Test Items, the CellTitre-Glo Luminescent Cell Viability Assay to measure ATP content will be performed according to the manufacturer's detailed instructions (Promega Corporation, Technical Bulletin No. 288, and Cell notes, Issue 10, 2004).

[0291] The results of the ATP depletion assay were corrected for background luminescence and expressed as a percentage of the vehicle control value using Microsoft Excel software. The results were graphed as ATP content (percentage of appropriate control) versus Test Item concentration (CXR1002).

Results

UO126 (FIG. 61-64)

[0292] Use of the CXR1002/UO126 combination compared to CXR1002 alone revealed increased sensitivity in the following cell lines:

TABLE 21

		IC_{50}	
Cell line	Tissue	CXR1002 (μM)	CXR1002 & U0126 (μM)
H	Sarcoma	817	453
OUMS-27	Sarcoma	821	410
PANC-1	Pancreas	>1000	897
BxPc3	Pancreas	381	247
TOV-21G	Ovarian	615	389
OV-90	Ovarian	714	329
CaCO2	Colon	704	285

[0293] It is clear from this data that concomitant inhibition of MEK1/2 may enhance the efficacy of CXR1002 and that the effect may be selective to certain cell lines and therefore tumour types.

LY294002 (FIG. 61-62)

[0294] LY294002 is a potent inhibitor of phosphoinositide 3-kinases. When used in conjunction with CXR1002 increased efficacy was noted in a select number of cell lines most notably the sarcoma cell line H.

DPQ (FIG. 65-66)

[0295] Poly(ADP-ribose) polymerase-1 (PARP-1) is a nuclear enzyme involved in DNA repair, replication and cell cycle. However, its overactivation leads to nicotinamide adenine dinucleotide and ATP depletion and cell death. Use of the PARP inhibitor, DPQ, in conjunction with CXR1002 led to increased sensitivity in the following cell lines: HPAFII and Capan2 (pancreas) and SW1353 (sarcoma). Again, the synergistic response with the two drugs was selective.

Conclusion

[0296] It is clear from this preliminary data that certain combinations of CXR1002 and the inhibitors UO126 or LY294002 or DPQ result in an increased level of cytotoxicity when compared to the use of CXR1002 alone. The exact mechanisms underpinning these observations remain to be elucidated. However, with regard to kinase pathways, the pathways (MAPK/PI3K/Akt) are known to form the core intracellular signalling routers in the stimulation of growth factors. Their expression, particularly that of PI3K/Akt, or that of their phosphorylated (activated) forms has been reported as a significant prognosis marker in sarcoma (Tomita, Y (2006)), gastric cancer (Cinti, C (2008)), pancreatic cancer (Chada, K S (2006)) and breast cancer (Park, S S (2007)). Therefore, inactivation of the PI3K/Akt or MAPK pathways should be effective as a specific chemotherapy against malignant tumours because of lower expression of activated forms in the surrounding tissues. In addition, these pathways play an essential role as survival signal pathways when cancer cells are exposed to a cellular stress. CXR1002 may cause cellular stress e.g. oxidative stress and therefore may activate certain stress-related responses. Therefore, use of inhibitors of these pathways might be expected to enhance the degree of cytotoxicity in cancer cell lines exposed to CXR 1002 and perhaps even in vivo.

[0297] Poly(ADP-ribose)polymerase (PARP) or poly (ADP-ribose)synthase (PARS) has an essential role in facilitating DNA repair, controlling RNA transcription, mediating cell death, and regulating immune response. In various cancer models, PARP inhibitors have been shown to potentiate radiation and chemotherapy by increasing apoptosis of cancer cells, limiting tumour growth, decreasing metastasis, and prolonging the survival of tumour-bearing animals. Again, it appears that use of PARP inhibitors in conjunction with CXR1002 potentiates cytotoxicity.

Example 8

ER Stress Effects of CXR1002

[0298] Investigation into the ER stress effects of CXR1007 were conducted by looking at whether CXR1002 induces expression of ER stress-regulated proteins and then splicing of XBPI MRNA upon CXR1002 induced ER stress.

Induction of Expression of ER Stress-Regulated Protein

[0299] Panc-1 (pancreatic tumour) cells were treated with vehicle control (lane 1), with 500 μM of CXR1002 for 4 h (lane 2), with 500 μM of CXR1002 for 1 day (lane 3), with 500 μM of CXR1002 for 2 days (lane 4), with 500 μM of CXR1002 for 3 days and with 500 μM of CXR1002 for 4 days.

[0300] Western blots were performed on the protein extracts (equivalent protein concentrations were loaded onto the gels). The antibodies used were for known ER stress regulated protein Bip/GRP78, CHOP/DDIT3, IRE1alpha,

TRB3 (Tribbles3), cleaved PARP (marker of apoptosis), and tubulin (loading control) (see FIG. **71**).

[0301] This showed that CXR1002 altered expression of ER stress-regulated proteins.

Splicing of XBP1 mRNA as an Indicator of ER Stress Inclusion

[0302] FIG. **72** shows the results of RT-PCR analysis of XBP1 mRNA splicing using RNA templates from CXR1002 treated cells. XBP1-u: unspliced form of XBPI; XBPI-s: spliced form of XBP1.

[0303] Panel (A) of FIG. **72** shows Panc-1 cells that were treated with CXR1002 for different time courses. 1. Control; 2. 500 μM/1 day; 3. 500 μM/2 days; 4. μM/3 days; 5. 500 μM/4 days; 6. μM/1 day; 7. 740 μM/2 days.

[0304] Panel (B) of FIG. **72** shows HepG2 cells that were treated with 300 μM of CXR1002 for different time courses 1. Control/1 day; 2. Control/2 days; 3. Control/4 days; 4. Tunicamycin for 24 h; 5. Tunicamycin for 6 h; 6. 300 μM/1 day; 7. 300 μM/2 days; 8. 300 μM/3 days.

[0305] Tunicamycin, 10 mg/mL. This is a control compound known to induce ER stress and XBP-1 splicing.

[0306] The RT-PCR analysis shows that XBP-1 splicing varies from predominately unspliced to spliced after treatment with CXR1002. XBP-1 is known to be spliced when ER stress is induced.

Example 9

[0307] PIM kinase activity after CXR1002 exposure. PIM kinase inhibition has been investigated for each of PIM-1, PIM-2 and PIM-3 kinase molecules.

PIM 1 (h)

[0308] The PIM-1 assay is performed using the Upstate IC_{50} Profiler Express™ service. In a final reaction volume of 25 μl, human recombinant PIM-1 (5-10mU) is incubated with 8 mM MOPS pH 7.0, 0.2 mM EDTA, 100 μM KKRNRTLTV, 10 mM MgAcetate and [γ-^{33}P-ATP] (specific activity approx. 500 cpm/pmol, concentration as required). After incubation for 40 minutes at room temperature, the reaction is stopped by the addition of 3% phosphoric acid solution. 10 μl of the reaction is then spotted onto a P30 filtermat and washed three times for 5 minutes in 75 mM phosphoric acid and once in methanol prior to drying and scintillation counting.

PIM-2 (h)

[0309] The PIM2 assay is performed using the Upstate IC_{50} Profiler Express™ service. In a final reaction volume of 25 μl, human recombinant PIM-2 (5-10 mU) is incubated with 8 mM MOPS pH 7.0, 0.2 mM EDTA, 300 μM RSRHSSYPAGT, 10 mM MgAcetate and [?-^{33}P-ATP] (specific activity approx. 500 cpm/pmol, concentration as required). After incubation for 40 minutes at room temperature, the reaction is stopped by the addition of 3% phosphoric acid solution. 10 μl of the reaction is then spotted onto a P30 filtermat and washed three times for 5 minutes in 75 mM phosphoric acid and once in menthanol prior to drying and scintillation counting.

PIM-3 (h)

[0310] The PIM-3 assay is performed using the Upstate IC_{50} Profiler Express™ service. In a final reaction volume of 25 μl, human recombinant PIM-3 (5-10 mU) is incubated with 8 mM MOPS pH 7.0, 0.2 mM EDTA, 300 μM RSRHSSYPAGT, 10 mM MgAcetate and [?-^{33}P-ATP] (specific activity approx. 500 cpm/pmol, concentration as required). After incubation for 40 minutes at room temperature, the reaction is stopped by the addition of 3% phosphoric acid solution. 10 μl of the reaction is then spotted onto a P30 filtermat and washed three times for 5 minutes in 75 mM phosphoric acid and once in menthanol prior to drying and scintillation counting.

Results

[0311] In each of the three PIM kinase assays, CXR 1002 shows inhibition of the kinase molecules.

Kinase	EC_{50}
PIM 1	40
PIM2	170
PIM3	240

Example 10

[0312] CXR1002 pharmacokinetics. PK sampling (repeat dose) in CXR1002 clinical trial

[0313] The methodology of this clinical trial is detailed in Example 5.

[0314] Plasma was collected at regular intervals (2, 3, 4 and 24 hrs post dose) every week from fasted patients (fasted minimum of 1 hour pre and post dose) administered weekly doses of CXR1002

Treatment (Cohort, Patients and Weekly Dose):

Cohort 2: Patient 004 (50 mg)

Cohort 3: Patients 005-007 (100 mg)

Cohort 4: Patients 008-010 (200 mg)

Cohort 5: Patients 011-014 (300 mg)

Cohort 6: Patients 015-017 (450 mg)

Cohort 7: Patients 018-024 (600 mg)

Cohort 8: Patients 025-028 (750 mg)

*Cohort 9: Patients 029-032 (950 mg)

Cohort 10: Patients 033-037 (1200 mg)

[0315] (*pt. 031 was not dosed)

[0316] FIGS. **74-78** show the results of the repeat dosing in terms of CXR2002 plasma levels.

[0317] Urine was collected over a 24 hour duration post each weekly dose and CXR1002 levels were measured in the total sample. FIG. **79** shows the urinary excretion (μg) of CXR1002 in 6 patients at 6 time points. FIG. **80** shows that the urinary excretion of CXR1002 is reflected in the pharmacokinetic profile of patient 29 with high levels of urinary excretion.

Results of Repeat Dose Pharmacokinetics:

[0318] As shown in FIGS. **74-78**, CXR1002 plasma concentration was cumulative and increased with both dose and duration of dosing. There was demonstrable dose equivalence (FIG. **75**). As shown in FIG. **79**, urinary excretion of CXR1002 increases with multiple doses and the pharmokinetic profile of CXR1002 changes to reflect urinary excretion (FIG. **80**).

Example 11

CXR1002 Effects on LDL and HDL

[0319] For detailed methodology, see Example 5.

Sample Selection and Rationale

[0320] Patients 004-036 were included in the analysis (*patients 34, 35 & 38 were not available for analysis. CXR1002 was administered (at dose increments; n=3-6) daily for a 6-week period. Plasma was collected and analysed for PK & PD effects. Data was initially grouped by dose and then re-grouped by PK (peak plasma exposure on wk 6). PD data: Plasma samples=baseline vs. wk 6 (peak plasma). Comparable graphs were plotted whether grouped by dose or drug exposure.

Data Analysis:

[0321] Individual patient raw data was captured and represented in graphs as % change from baseline (screening). Individual patient data (% baseline) was plotted in Prism and data was grouped according to PEAK plasma [CXR1002] at wk 6. Data represents either mean±SEM values or median, range+individual data points.

[0322] FIGS. **81** and **82** show the effect (% baseline) of 6 weeks of CXR1002 treatment on plasma High-density lipoprotein cholesterol (HDL-C) and Low-density lipoprotein cholesterol (LDL-C) levels respectively for patients grouped by peak plasma exposure.

[0323] The data suggests an effect of CXR1002 on LDL (i.e. lowering effect) but not HDL (i.e. CXR1002 lowers 'bad' cholesterol but 'good' cholesterol remains unchanged). This effect is entirely predicted from the animal data and suggests a possible use in patients with conditions such as high cholesterol and hyperlipidemia.

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1. A composition comprising between 10 mg and 2000 mg of an active ingredient per dosage unit, wherein the active ingredient is perfluorooctanoic acid (PFOA) or a salt, derivative or variant thereof.

2. The composition of claim **1**, wherein the PFOA is ammonium perfluorooctanoic acid (APFO).

3.-**6**. (canceled)

7. The composition of claim **1**, wherein the composition further comprises a pharmaceutically acceptable excipient, diluent, carrier or filler.

8.-**10**. (canceled)

11. A method of treating cancer comprising administering to a patient in need thereof an effective amount of a composition as defined in claim **1**, in a single dosage at a frequency of twice per week or less.

12. The method of claim **11**, wherein the effective amount is between 1 mg/kg and 7 mg/kg

13. (canceled)

14. The method of claim **11**, wherein the single dosage is administered at a frequency of once per six weeks or less.

15. The method of claim **11**, wherein the single dosage is between 50 mg and 1200 mg and is administered at a frequency of once per week or less.

16.-**19**. (canceled)

20. The method of claim **11**, wherein the cancer pancreatic cancer, ovarian cancer, breast cancer, prostate cancer, liver cancer, chondrosarcoma, lung cancer, head and neck cancer, colon cancer, sarcoma, leukaemia, lymphoma, kidney cancer, thyroid cancer or brain cancers, including glioblastoma.

21. (canceled)

22. The composition of claim **1**, further comprising a further chemotherapeutic agent.

23. The composition of claim **22**, wherein the further chemotherapeutic is selected from Doxorubicin, Gemcitabine, Roscovitine, Rapamycin, 5-FU, PARP inhibitors, kinase inhibitors, including PIM kinase inhibitors and MAP kinase inhibitors, Hsp90 inhibitors, including Geldanamycin, proteasome inhibitors, including Bortezomib, HDAC inhibitors or prodrugs thereof.

24. The composition of claim **22**, wherein the further chemotherapeutic is present in an individually effective dose.

25. The composition of claim **22**, wherein the further chemotherapeutic is present in a lower than individually effective dose.

26.-**29**. (canceled)

30. A therapeutic system for the treatment of cancer comprising a combination of components which are (i) a composition as defined in claim **1**; and (ii) a further chemotherapeutic agent, wherein components (i) and (ii) are provided for the use in the treatment of cancer and components (i) and (ii) are administered in combination with one another.

31. The therapeutic system of claim **30**, wherein administration of component (i) precedes administration of component (ii).

32. The therapeutic system of claim **30**, wherein administration of component (ii) precedes administration of component (i).

33. The therapeutic system of claim **30**, wherein administration of component (i) occurs at the same time as administration of component (ii).

34. The therapeutic system of claim **30**, wherein the further chemotherapeutic is Doxorubicin, Gemcitabine, Geldanamycin, Roscovitine, Rapamycin, 5-FU, PARP inhibitors, kinase inhibitors, including MAP kinase inhibitors, or prodrugs thereof, and wherein the cancer is selected from pancreatic cancer, ovarian cancer, breast cancer, prostate cancer, liver cancer, chondrosarcoma, lung cancer, head and neck cancer, colon cancer, sarcoma, leukaemia, lymphoma, kidney cancer, thyroid cancer and brain cancers such as glioblastoma.

35.-**41**. (canceled)

42. A kit of parts comprising:

(i) a composition as defined in claim **1**; and

(ii) a further chemotherapeutic agent.

43. The kit of claim **42** further comprising:

(iii) means of administering (i) and (ii) to a patient, wherein the administration may be at the same time or in succession.

44. The kit of claim **42**, wherein the further chemotherapeutic agent is Doxorubicin, Gemcitabine, Roscovitine, Rapamycin, 5-FU, PARP inhibitors, kinase inhibitors, including PIM kinase inhibitors and MAP kinase inhibitors, Hsp90 inbhibitors, including Geldanamycin, proteasome inhibitors, including Bortezomib, HDAC inhibitors or prodrugs thereof.

45.-**48**. (canceled)

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